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Supplementary Materials

Green access to chiral Vince lactam in a buffer-free aqueous system using a newly identified substrate-tolerant (−)-γ-lactamase

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Table S1 Active γ-lactamase genes identified by genome mining and primers used in this study

Enzyme	Entry	Organism	Primers
*Pp*GL	Q88CC8	*Pseudomonas putida*	F: CGCGGATCCATGAGCACGTTCGTTACC R: CCCAAGCTTTTACCGCTGAAGGAACGC
*Sv*GL	D9XDN2	*Streptomyces viridochromogenes*	F: CGGAATTCATGCCGTACATCACCGTG R: CCCAAGCTTTCACTTCTCCAGGAAGGC
*Gd*GL	B5ZI99	*Gluconacetobacter diazotrophicus*	F: CGCGGATCCATGTCGCATTTCACGACG R: CCCAAGCTTTCAGTGTTTCAGGAATTCC
*Gt*GL	H5UHP0	*Gordonia terrae*	F: CGCGGATCCATGGGCTACATCAAAGTC R: CCCAAGCTTTCACTTCTGAACGAACCC
*Se*GL	K0K3C7	*Saccharothrix espanaensis*	F: CGCGGATCCATGCCGTACATCACCGTG R: CCCAAGCTTTCAGTCGGCGAGGAAGCT
*Cf*GL1	F4GZY6	*Cellulomonas fimi*	F: CGCGGATCCATGCCGTACATCACGAGCA R: CCCAAGCTTTCAGGCGGAGAGGAAGTC
*Ac*GL	B8HFZ6	*Arthrobacter chlorophenolicus*	F: CGCGGATCCATGGCTTTCATCACCGTTG R: CCCAAGCTTCTACTTGGCCAGGAAGCC
*Ev*GL	L0G171	*Echinicola vietnamensis*	F: CGCGGATCCATGCCATTTTTGATCAATG R: CCGCTCGAGTTATTTTTTCAGAAAATCCAG
*Bm*GL	D5DK94	*Bacillus megaterium*	F: CGCGGATCCATGGCAAAAATTAATGTAGG R: CCCAAGCTTTTAGTCTCTTAAAAATGACAG
*Mp*GL	F5XPN5	*Microlunatus phosphovorus*	F: CGCGGATCCATGCCCTTCATCACCGTC R: CCCAAGCTTTCAACTCGCCAAGAAGCC
*Cf*GL2	F4H619	*Cellulomonas fimi*	F: CGCGGATCCATGGGAACGATCACGACCA R: CCCAAGCTTTCAGGAGCGGAGGAACTC

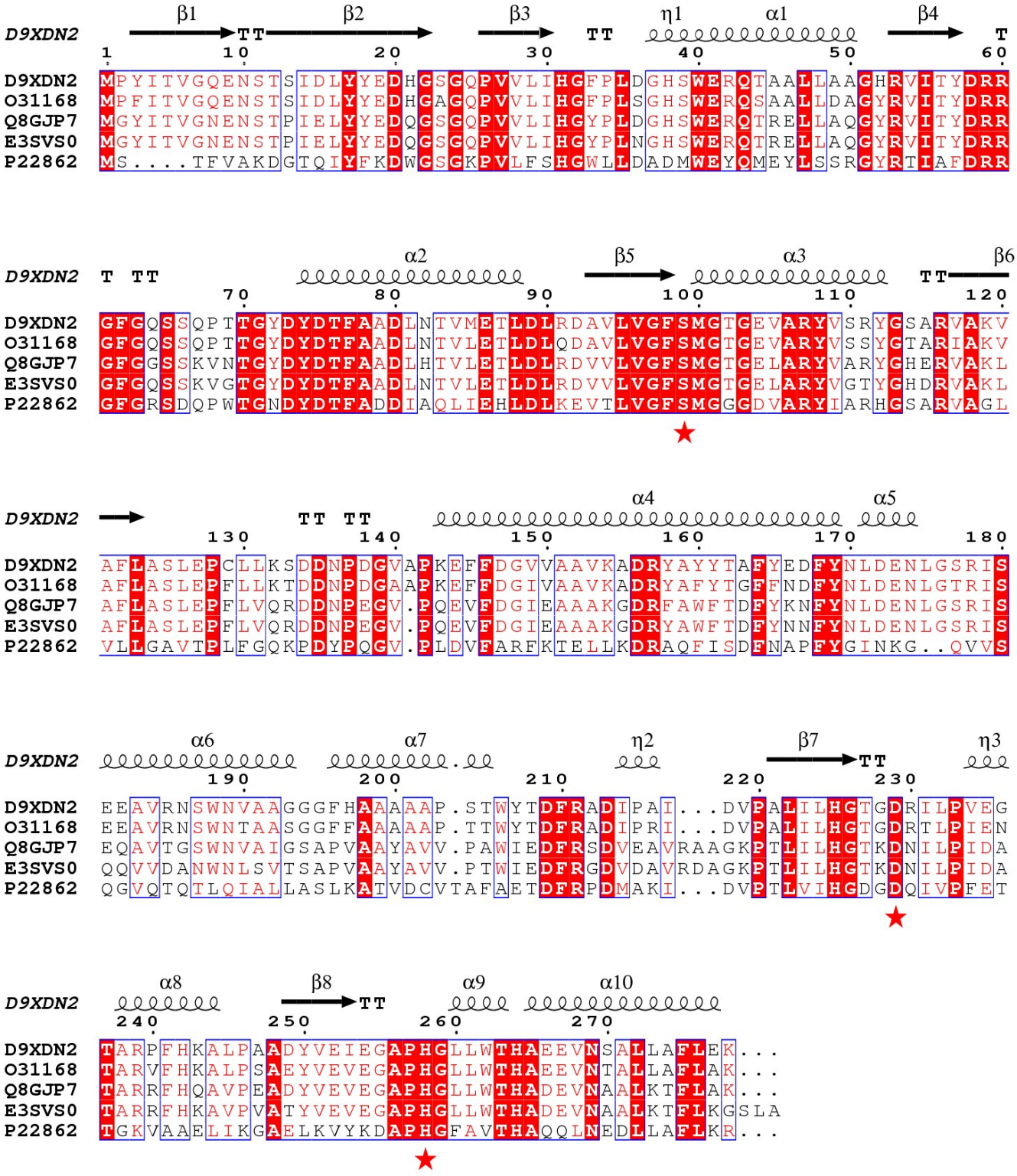

Fig. S1 Multiple sequences alignment of *Sv*GL with some related enzymes. *Sv*GL from *Streptomyces viridochromogenes* (D9XDN2), non-heme chloroperoxidase from *Streptomyces aureofaciens* (O31168), (−)-γ-lactamase from *Aureobacterium* sp. (Q8GJP7), (−)-γ-lactamase from *Microbacterium hydrocarbonoxydans* (E3SVS0), and arylesterase from *Pseudomonas fluorescens* (P22862). The catalytic triad is indicated by the symbol (★).

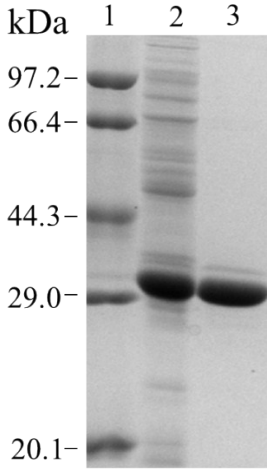

Fig. S2 SDS-PAGE analysis of purified *Sv*GL. Lane 1, protein markers; Lane 2, crude cell-free extract; Lane 3, purified enzyme.

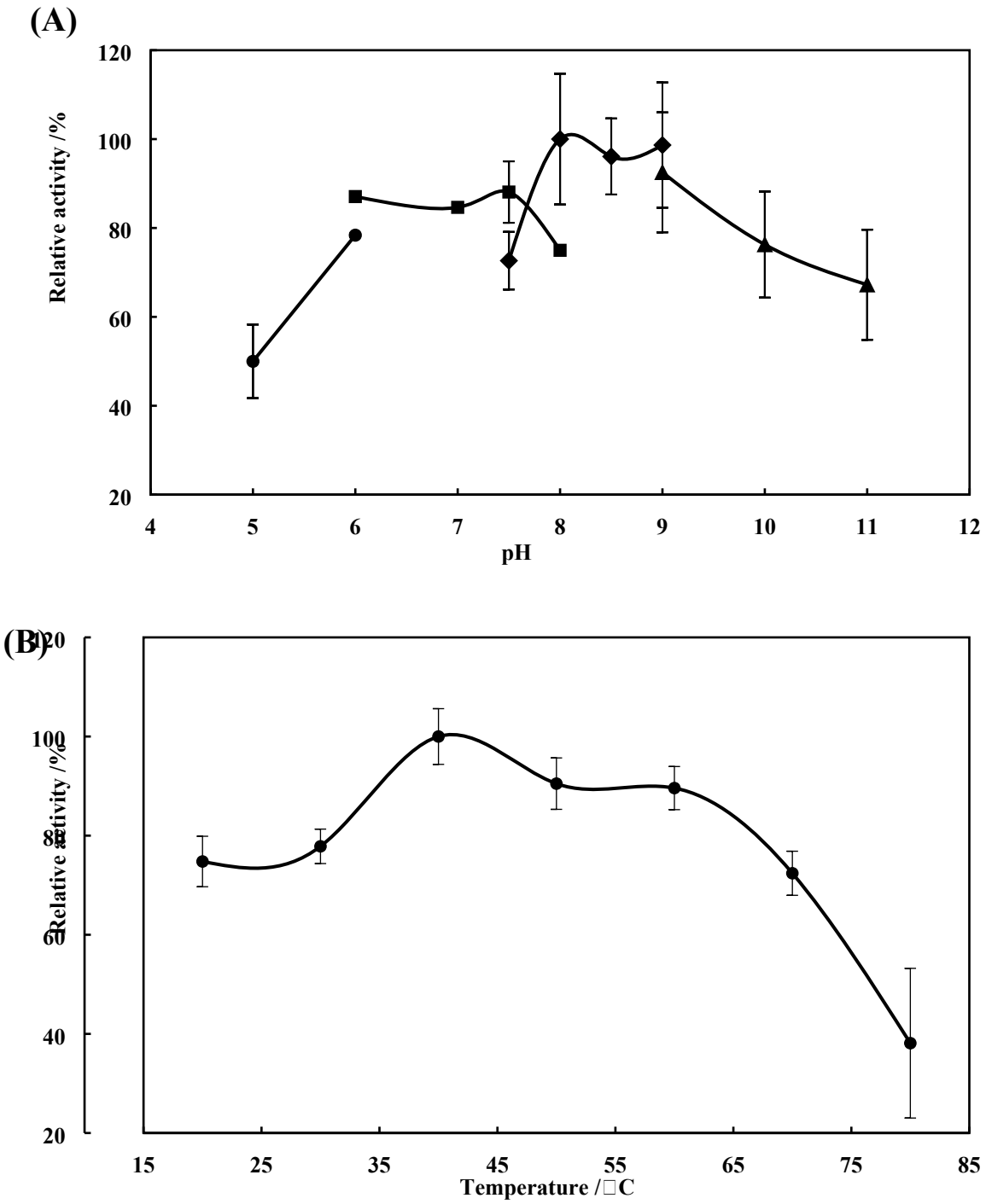

Fig. S3 (**A**) Effect of pH on *Sv*GL activity. Enzyme assay was performed in different buffers (100 mM) within a pH range of 6.0–10.0 (sodium citrate buffer, 5.0–6.0 (ஜ); potassium phosphate, 6.0–8.0 (O); Tris–HCl, 7.5–9.0 (◆); Gly–NaOH, 9.0–11.0 (▲)) at 30°C. (**B**) Effect of temperature on *Sv*GL activity. Relative activity was expressed as a percentage of the maximum activity under the experimental conditions.

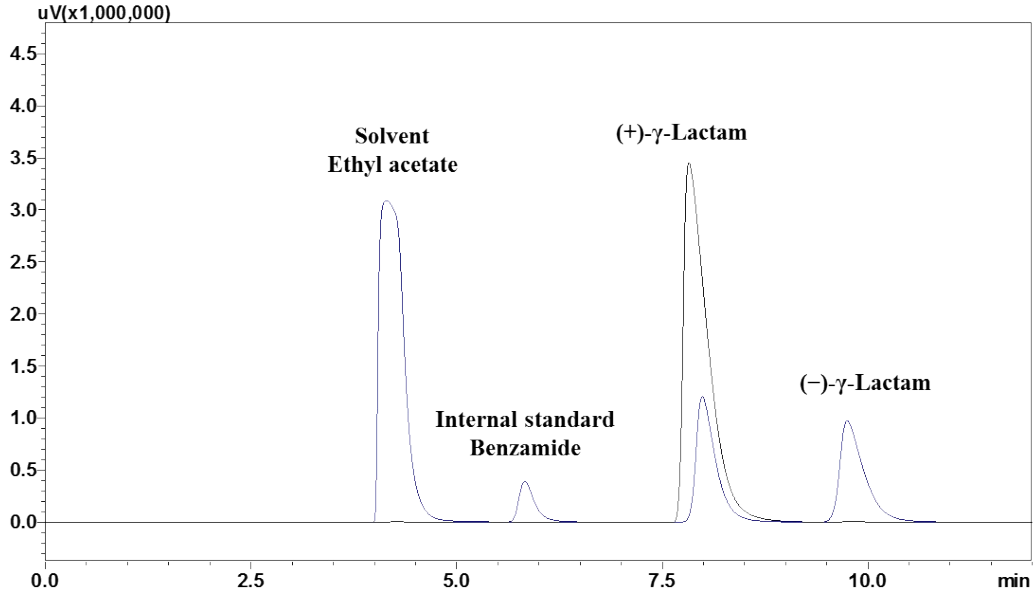

Fig. S4 Chiral HPLC analysis of (+)-γ-lactam synthesized by *Sv*GL. Blue line: Standard *rac*-γ-lactam, black line: The recovered (+)-γ-lactam product synthesized by *Sv*GL.

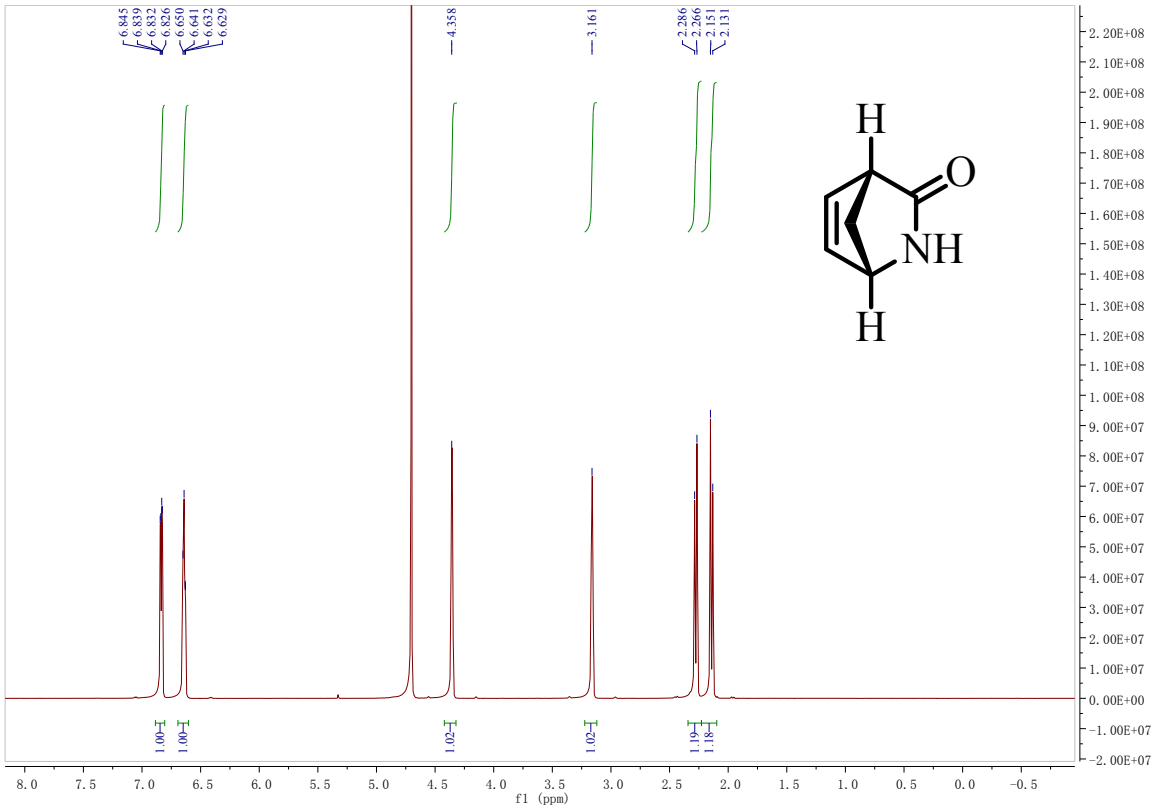

Fig. S5 ^{1}H NMR spectra of (+)-γ-lactam synthesized by *Sv*GL.

^{1}H NMR (400 MHz, D_2O): δ = 2.14 (1H, d, *J* = 8 Hz, −C*H*H−), δ = 2.28 (1H, d, *J* = 8 Hz,−CH*H*−), δ = 3.16 (1H, s, −COC*H*CH=), δ = 4.36 (1H, s, −NHC*H*CH=), δ = 6.60−6.69 (1H, m, −C*H*=CH−), δ = 6.83 (1H, dd, J_1 = 5.2 Hz, J_2 = 2.4 Hz,−C*H*=CH−).