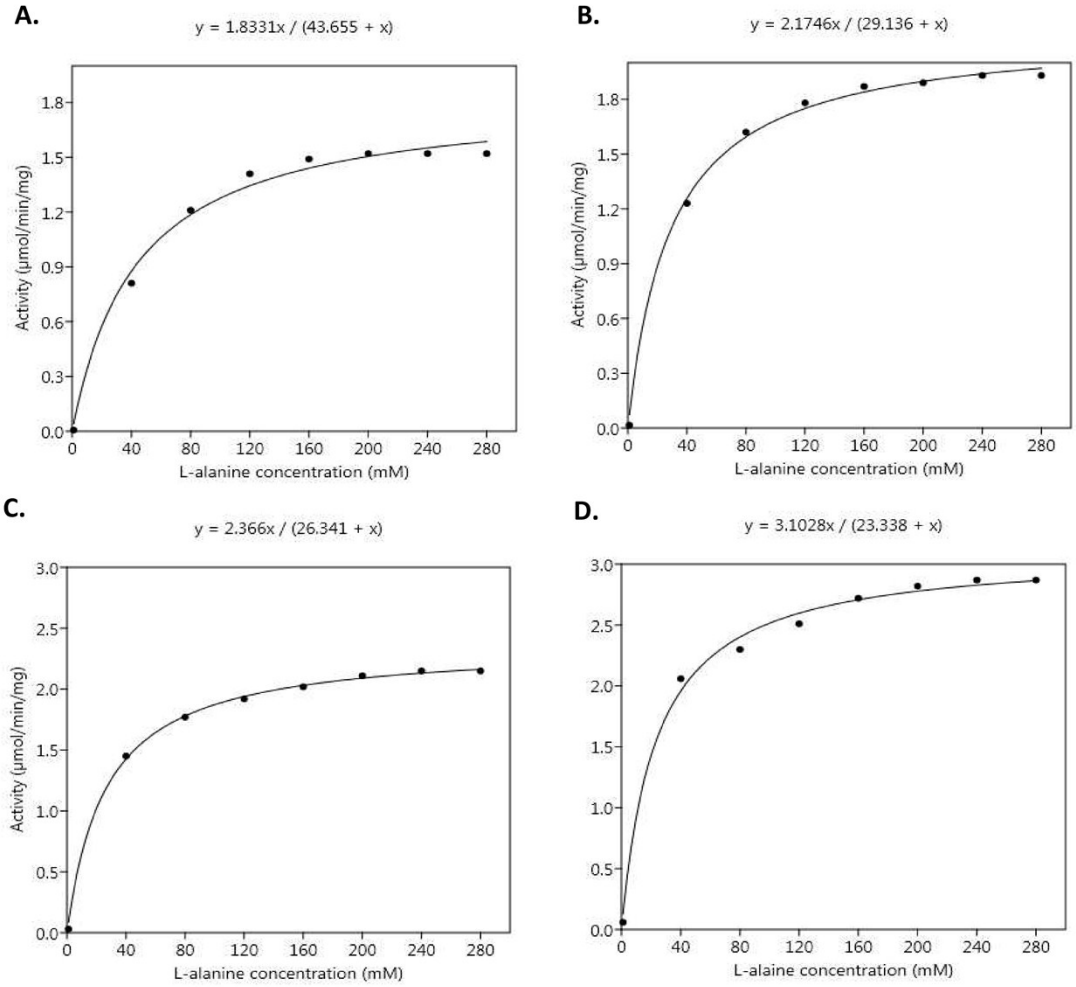

Figure S1: Michaelis-Menten graphs and curve fits using L-alanine as a substrate. **A**. Wild type pm1; **B**. pm1ep1; **C.** pm1ep2; & **D.** pm1ep3.

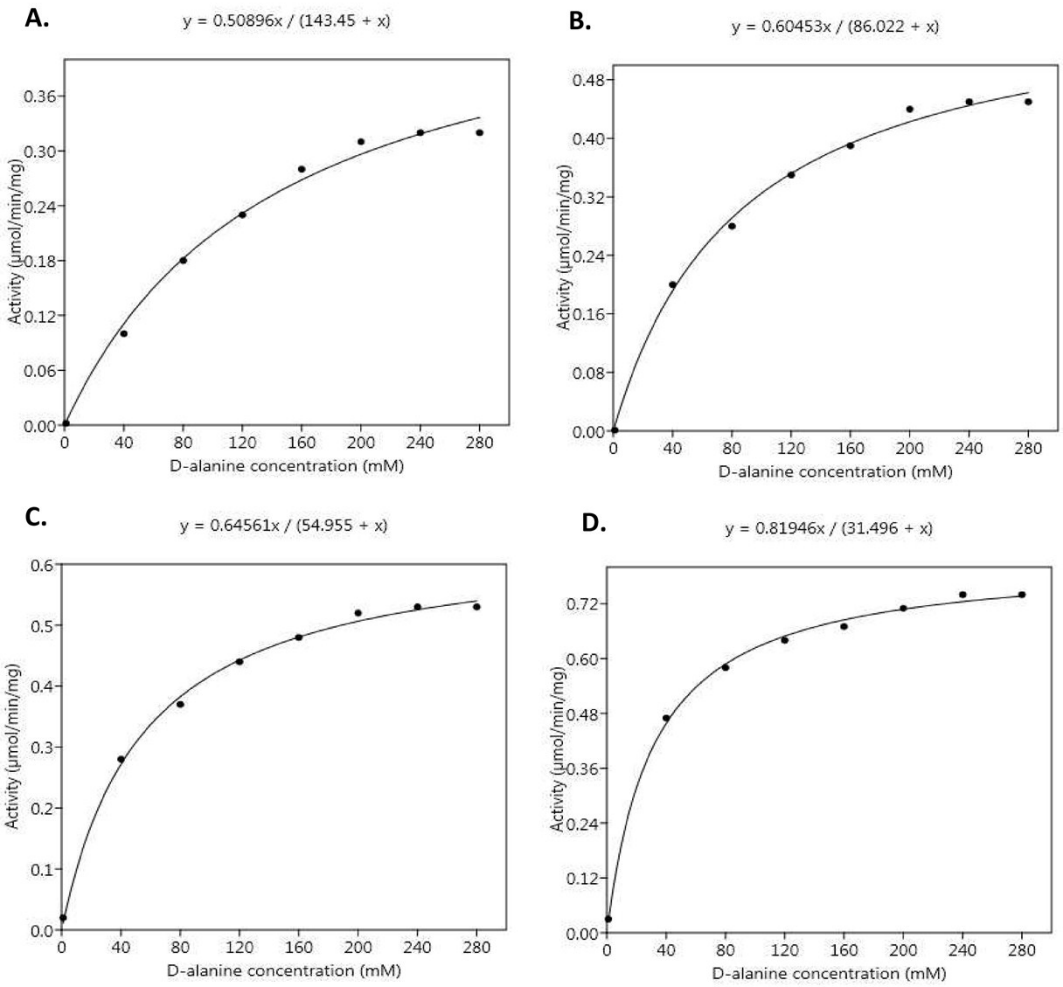

Figure S2: Michaelis-Menten graphs and curve fits using D-alanine as a substrate. **A**. Wild type pm1; **B**. pm1ep1; **C.** pm1ep2; & **D.** pm1ep3.

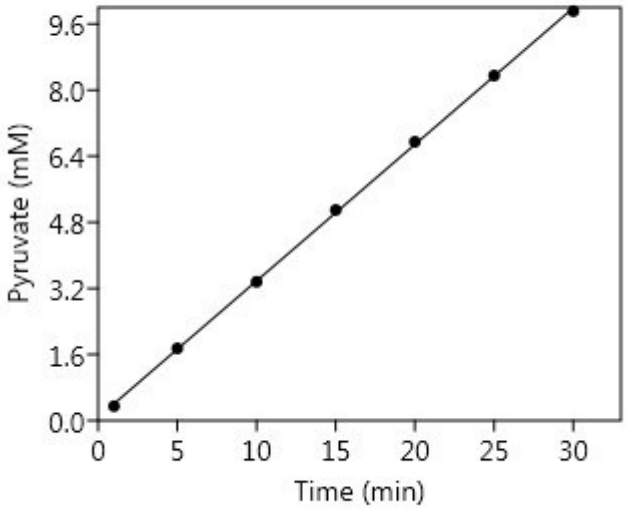

Figure S3: Initial pyruvate production by recombinant strain containing pm1ep3.

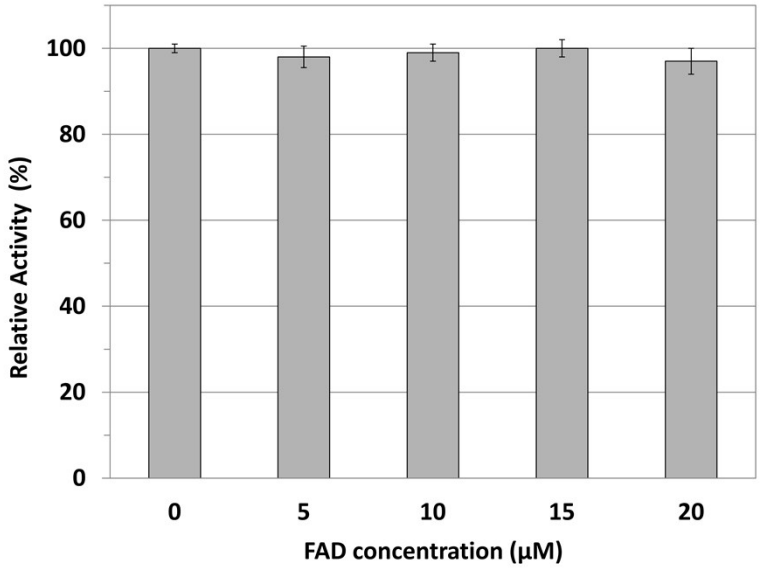

Figure S4: Effect of external concentration of FAD on the enzyme's activity. The highest activity of the pm1 (1.83 µmol·min^{-1}·mg^{-1}) was defined as 100%.

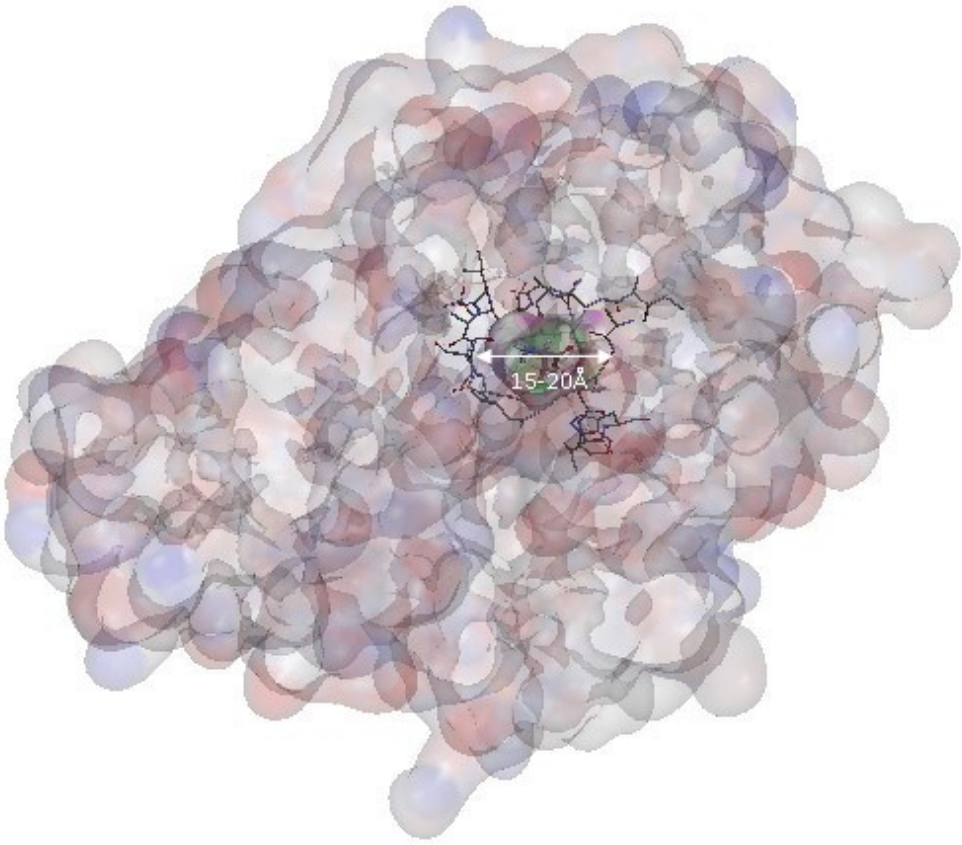

Figure S5: Substrate entrance site in pm1 (surface representation).

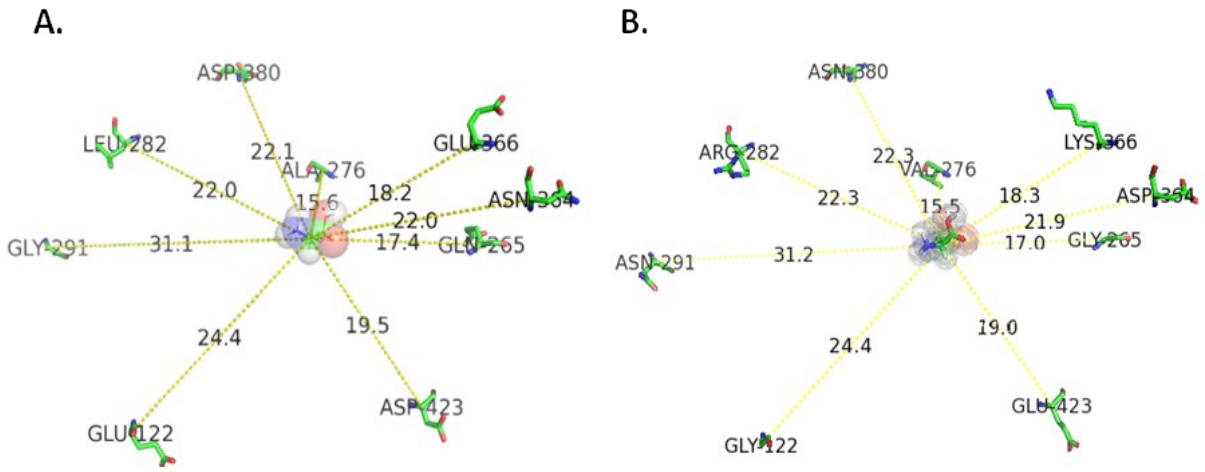

Figure S6: Molecular distances of alanine (substrate) from the mutant sites. **A**. in the wild type pm1; **B**. in the mutant pm1ep3.

Table S1: Primers used in this study

Primer name	Nucleotide sequence (5'-3')
	Primer used for epPCR
Pm1_F1	CGCGGATCCATGGCAATAAGTAGAAGAAAATTTA
Pm1_R1	TCCGAGCTCTTAGAAACGATACAGACTAAATGGT
	Primer used for genes knockout and verification
*cyc*A-knockF	ATGGTAGATCAGGTAAAAGTCGTTGCCGATGATCAGGCTCCGGCTGAACAATTCCGGGGATCCGTCGACC
*cyc*A-knockR	TTATTTCCGCAGTTCAGCAGCCCGCTTCTTACCAATAAACAACCAGCCCATGTAGGCTGGAGCTGCTTCG
*cyc*A-verF	GCCTGAACAACACAGACAGGTACAGGAAGA
*cyc*A-verR	CTGGATGGCATTGCGCCATCCAGCATGATA
*ama*P-knockF	ATGATGAACACGGAAGGTAATAACGGTAACAAACCTCTCGGTCTATGGAAATTCCGGGGATCCGTCGACC
*ama*P-knockR	TTATACGGTTTTATTGCGCTTCATGACCATTGCCACAATAAGGCTGAGTATGTAGGCTGGAGCTGCTTCG
*ama*P-verF	TCGGTCGCTAAGCAACTCGGCTATAACGTG
*ama*P-verR	ACCGCCACCACAATAATACAGGAAGTACTG
*lld*P-knockF	ATGAATCTCTGGCAACAAAACTACGATCCCGCCGGGAATATCTGGCTTTCATTCCGGGGATCCGTCGACC
*lld*P-knockR	TTAAGGAATCATCCACGTTAAGACATAAGCCTGAAGCGTGGTGATCACGCTGTAGGCTGGAGCTGCTTCG
*lld*P-verF	CATTACACGATGTGCGTGGACTCCAGGAGA
*lld*P-verR	CGGCAACCTCGTCTGACAGGCGTCTGGGTA

Table S2: Thermodynamic parameter for L-alanine binding to wild type and its mutants.

Enzyme	K_d (mM)	ΔH^o (kcal.mol^{-1})	ΔG^o (kcal.mol^{-1})
Wild type (Pm1)	15.21 ± 0.34	–9.53 ± 0.15	–7.08 ± 0.07
Pm1ep1	11.93 ± 0.12	–11.09 ± 0.21	–6.45 ± 0.11
Pm1ep2	9.15 ± 0.11	–12.14 ± 0.08	–5.76 ± 0.08
Pm1ep3	6.76 ± 0.09	–13.27 ± 0.14	–4.97 ± 0.03

K_d and ΔH^o were obtained from fit to a binding isotherm, while ΔG^o was calculated from $\Delta G^o = RT\ln K_d$. Each value represents the mean of three or more independent ITC experiments.

Table S3: K_m values of different amino acid deaminases/oxidases.

Enzyme's name	Origin	Substrate	K_m (mM)	References
L-amino acid deaminase	*Proteus mirabilis*	L-Phenylalanine	31.55	35
L-amino acid deaminase	*Proteus mirabilis*	L-Phenylalanine	26.2	S1
L-amino acid deaminase	*Proteus myxofaciens*	L-Phenylalanine	2.28	S2
L-Amino Acid Oxidases	*Proteus rettgeri*	L-Lysine	23.2	S3
L-amino acid deaminase	*Proteus vulgaris*	L-Methionine	305.0	S4

S1: Y. Hou, G. S. Hossain, J. Li, H. D. Shin, L. Liu, and G. Du, *Appl. Microbiol. Biotechnol.*, 2015, **99**, 8391-8402. S2: D. P. Pantaleone, A. M. Geller, and P. P. Taylor, *J. Mol. Catal. B: Enzymol.*, 2001, **11**, 795–803. S3: J. A. Duerre, and S. Chakrabarty, *J. Bacteriol.*, 1975, **121**, 656-663. S4: G. S. Hossain , J. Li , H. D. Shin, G. Du, M. Wang, L. Liu, and J. Chen, *PLoS One*, 2014, **9**, e114291.