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The role of surface exposed lysine in conformational stability and functional properties of lipase from staphylococcus family

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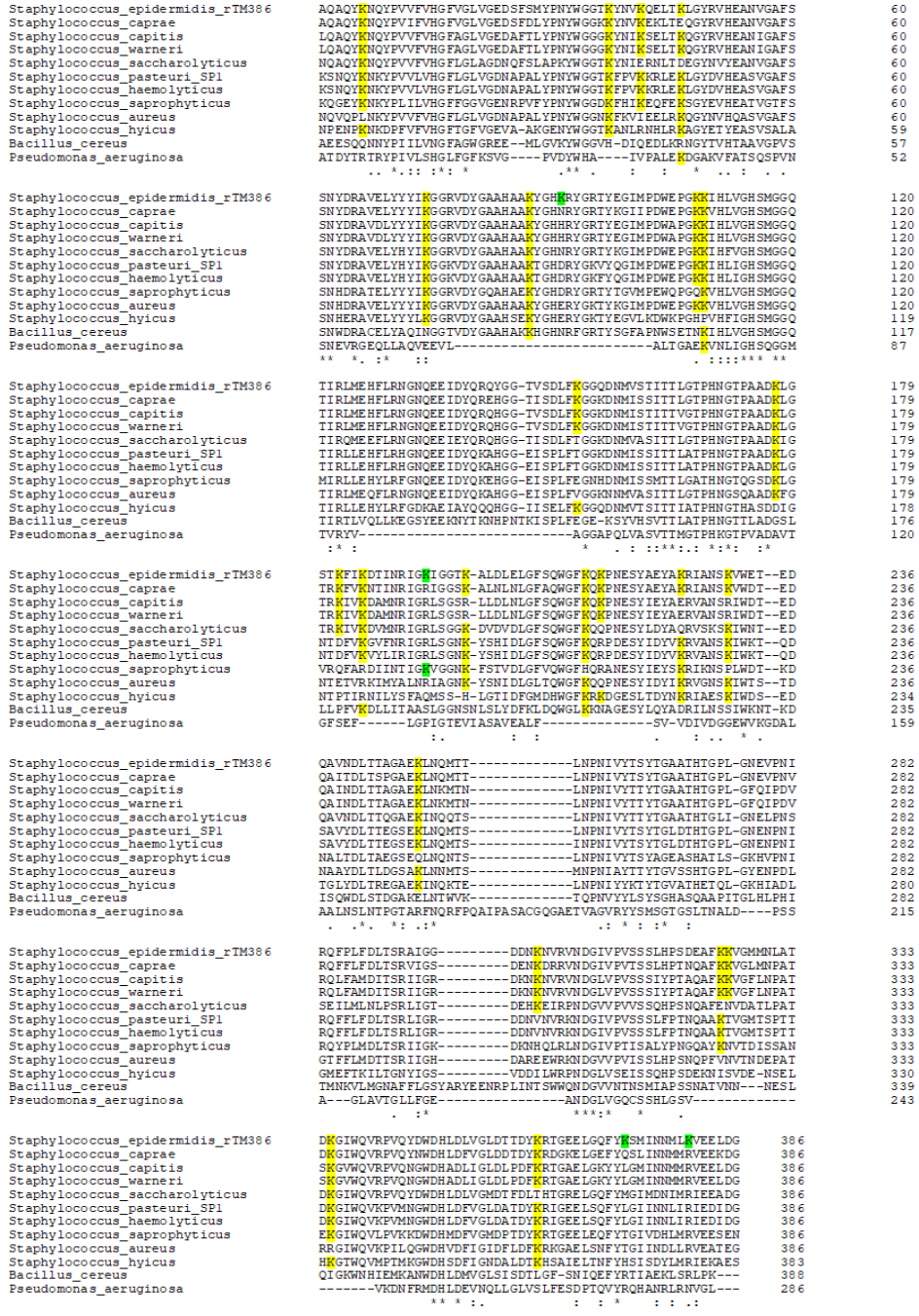

Figure S1. Multiple sequence alignment of WT lipase to other Staphylococcal lipase (Family I.6) and other genera of *Bacillus* and *Pseudomonas*. The highlighted in yellow colour showed lysine residue (highly conserved or non-conserved) across the various species of *Staphylococcus*; and *Bacillus* and *Pseudomonas* genera.

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WT      ------------------------------------------AQAQYKNQYPVVFVHG  16
2HIH    MARIRARGSSRVDVPKENTTAQNKFTSQASDKKPTVKAAPEAVQNPENPKNKDPFVFVHG  60
                                                  : **: *.*****

WT      FVGLVGEDSFSMYPNYWGGTKYNVKQELTKLGYRVHEANVGAFSSNYDRAVELYYYIKGG  76
2HIH    FTGFVGEVA-AKGENYWGGTKANLRNHLRKAGYETYEASVSALASNHERAVELYYYLKGG  119
        *.*:*** : :   ******* *:::.* * **..:**.*.*::**::********:***

WT      RVDYGAAHAAKYGHKRYGRTYEGIMPDWEPGKKIHLVGHSMGGQTIRLMEHFLRNGNQEE  136
2HIH    RVDYGAAHSEKYGHERYGKTYEGVLKDWKPGHPVHFIGHSMGGQTIRLLEHYLRFGDKAE  179
        ********: ****:***:****:: **:**: :*::*********:**:** *:: *

WT      IDYQRQYGGTVSDLFKGGQDNMVSTITTLGTPHNGTPAADKLGSTKFIKDTINRIGKIGG  196
2HIH    IAYQQQHGGIISELFKGGQDNMVTSITTIATPHNGTHASDDIGNTPTIRNILYSFAQMSS  239
        * **:*:** :*:**********::***:.****** *:*.:*.*  *:: :  :.::..

WT      TKALDLELGFSQWGFKQKPNESYAEYAKRIANSKVWETEDQAVNDLTTAGAEKLNQMTTL  256
2HIH    -HLGTIDFGMDHWGFKRKDGESLTDYNKRIAESKIWDSEDTGLYDLTREGAEKINQKTEL  298
         :   :::*:.:****:* .** ::* ****:**:*::** .: ***  ****:** * *

WT      NPNIVYTSYTGAATHTGPLGNEVPNIRQFPLFDLTSRAIGGDDNKNVRVNDGIVPVSSSL  316
2HIH    NPNIYYKTYTGVATHETQLGKHIADLGMEFTKILTGNYIGSVDDILWRPNDGLVSEISSQ  358
        **** *.:***.***   **:.: ::       **.. **. *:   * ***:*   **

WT      HPSDEAFKKVGMMNLATDKGIWQVRPVQYDWDHLDLVGLDTTDYKRTGEELGQFYKSMIN  376
2HIH    HPSDEKNISVD-ENSELHKGTWQVMPTMKGWDHSDFIGNDALDTKHSAIELTNFYHSISD  417
        *****   .*.  *   .** *** *.  .*** *::* *: * *::. ** :**:*: :

WT      NMLKVEELDG----        386
2HIH    YLMRIEKAESTKNA        431
         ::::*: :.
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Figure S2. Sequence alignment of WT lipase and the template, *S. hyicus* lipase (PBD ID: 2HIH). The conserved pentapeptide region (GXSXG) is shown in the red box. Symbols are indicated as follows: (*) fully conserved residue; (:) conservation of strong groups; and (.) conservation of weak group.

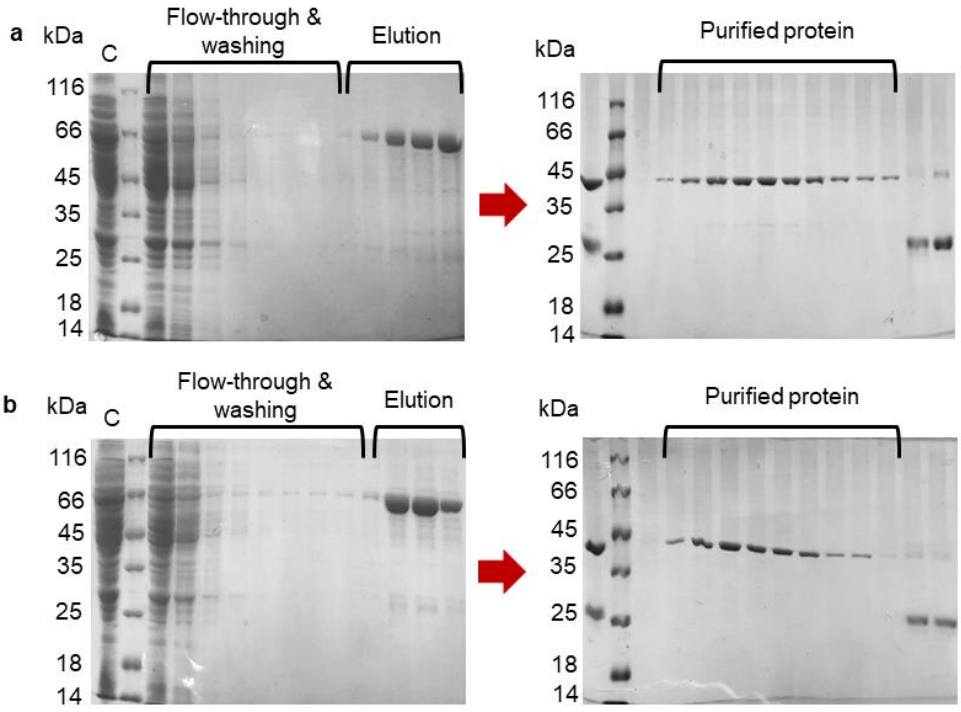

Figure S3. SDS-PAGE (12%) analysis of two-steps purification of **(a)** K325G and **(b)** K91A/K325G. Symbols denote (C) crude enzyme, flow-through of impurities and washing step fractions. The elution fractions represented GST-tagged mutant lipases (~69 KDa). GST tag was cleaved by PreScission Protease. Purified protein denotes purified mutant lipases (~43 KDa). GST tag was collected into later fractions (~26 kDa). A standard protein marker (Fisher Thermo Scientific, USA) was utilised.

Table 1. Predicted structure validation of WT and mutant lipases based on Ramachandran plot and Errat2.

Validation tools	The structure quality						
	WT	Single mutants			Double mutants		
		K91A	K177A	K325G	K91A/ K177A	K91A/ K325G	K177A/ K325G
Ramachandran Plot							
a) Most favored region	90.6%	90.9%	92.7%	92.1%	87.2%	92.1%	92.1%
b) Additional allowed region	9.1%	8.8%	6.4%	7.0%	11.9%	7.3%	7.3%
c) Generously allowed region	0.3%	0.3%	0.9%	0.9%	0.9%	0.3%	0.6%
d) Disallowed region	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%
Errat2 (Overall quality factor)	97.60	98.39	99.73	98.93	92.61	95.21	96.78

Table S2. QMEAN analysis of WT and the mutants. The analysis of QMEAN Z-scores consist of linear combination from six structural descriptors as scoring function to show the overall structure quality for each of the enzymes. Another four Z-scores of individual analysis were reported.

Lipases	QMEAN	C−β	All Atom	Solvation	Torsion
WT	−0.64	−0.48	−1.04	−0.41	−0.56
K91A	−0.46	−0.46	−1.27	−0.49	−0.19
K177A	−0.84	−0.87	−0.91	−0.38	−0.61
K325G	−0.83	−0.72	−0.90	−0.21	−0.65
K91A/K177A	−1.68	−0.85	−1.30	−0.94	−1.27
K91A/K325G	−0.66	−1.02	−1.08	−0.70	−0.33
K177A/K325G	−0.64	−0.82	−1.22	−0.65	−0.34