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Supporting information for article:

**Crystallographic analysis of the *Staphylococcus epidermidis*
lipase involved in esterification in aqueous solution**

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S1. Materials and methods

S1.1. Enzyme kinetic characterization

Enzyme kinetic assays of purified rGehC were done using *p*-NP esters under optimum pH and temperature condition (Chang *et al.*, 2000). The *p*-NP esters of various chain-length fatty acids (*p*-NP butyrate, C4; *p*-NP caprylate, C8; *p*-NP caprate, C10; *p*-NP myristate, C14) were determined by measuring the amount of *p*-nitrophenol released. The catalytic rate constant k_{cat} (s^{-1}) was calculated from the initial velocity according to the equation $k_{\text{cat}} = V_{\text{max}} / [\text{E}]$.

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rGehC .....MARIRARGSSRDVPKENTTAQNKFTSQASDKKPTVKA
2HIH .....
1KU0 .....
5AH1 MAEPKAQGTQKVESSTTKKEVKDAEETIKIPTLEDIDNLDISAEVVKSEEDINKMPLKF
4IIP .....

      1      10      20      30      40      50
rGehC .....MEQKQYKNDIILVHGFNGFTDDINPSVLTHYWGCDKMNIRQDLKENGCEA
2HIH A...PEAVQNPNPNKNDIIVVHGFNGFTGVGEVAAKG.ENYWGCTKANLRNHKRAAGYET
1KU0 .....ASPRANDAIIVLVHGFNGFTGVGEVAAKG.ENYWGCTKANLRNHKRAAGYET
5AH1 PVEFPVNTSRISIIIGNNYIIVLVHGFNGFTGVGEVAAKG.ENYWGCTKANLRNHKRAAGYET
4IIP .....ADNYAAIIVLVHGFNGFTGVGEVAAKG.ENYWGCTKANLRNHKRAAGYET

      60      70      80      90     100     110
rGehC YEASISAFGSNYDRAVELYXYIKGGRVDYGAHAHAKYGHERYGKTYEGVYKDWKPGOKIH
2HIH YEASVSALASNNHERAVELYXYIKGGRVDYGAHAHAKYGHERYGKTYEGVYKDWKPGOKIH
1KU0 YTLAVGPLSSNWDRACEAYALVGGTVDYGAHAHAKYGHERYGKTYEGVYKDWKPGOKIH
5AH1 YTLAVGPLSSNWDRACEAYALVGGTVDYGAHAHAKYGHERYGKTYEGVYKDWKPGOKIH
4IIP YVANLSGFQSD.....DGNRGRGEQLAVVKTIVLAATGATKVN

      120     130     140     150     160     170
rGehC LVGHSMSGGQIRLEELLRHGNPEEVEYQKQH.GGEISPLYQGGHDNMVSSITTLQTPHN
2HIH FIGHSMGGQIRLEELLRHGNPEEVEYQKQH.GGEISPLYQGGHDNMVSSITTLQTPHN
1KU0 LIAHSQGGQIRLEELLRHGNPEEVEYQKQH.GGEISPLYQGGHDNMVSSITTLQTPHN
5AH1 LIGHSMGGQIRLEELLRHGNPEEVEYQKQH.GGEISPLYQGGHDNMVSSITTLQTPHN
4IIP LVGHSMSGGQIRLEELLRHGNPEEVEYQKQH.GGEISPLYQGGHDNMVSSITTLQTPHN

      180     190     200     210     220
rGehC GTHASDLIGNEAIVRQLAY....DVGKMYGNKDSRVDFGLEHWGLKQKPNESYIQYVX
2HIH GTHASDLIGNEAIVRQLAY....DVGKMYGNKDSRVDFGLEHWGLKQKPNESYIQYVX
1KU0 GTTLLSDLMMPAKD.LISYTFGVLL.GTITGKNKLFSSIIYDLKLDQWGLKQKPNESYIQYVX
5AH1 GTTLLSDLMMPAKD.LISYTFGVLL.GTITGKNKLFSSIIYDLKLDQWGLKQKPNESYIQYVX
4IIP VNVFGLTSSSNNTNQDALAAIKTLTAAQAATYNQNYPSAGLSAGPSCQTGAPTETVGGN

      230     240     250     260     270     280
rGehC RVONSKLWKS.K.SGLHDLTRGATDLNRKRTSLNENIYVKTYYGESHKTLA.GKQKADL
2HIH RIAESKIWDSE.DTGLYDLTRGATDLNRKRTSLNENIYVKTYYGESHKTLA.GKQKADL
1KU0 RLKRSVWVST.DIARYLDSVSGATDLNRKRTSLNENIYVKTYYGESHKTLA.GKQKADL
5AH1 RVLDSSNWNSTKDIAIYDLSGATDLNRKRTSLNENIYVKTYYGESHKTLA.GKQKADL
4IIP THLLYSWAGAIQPTISVGVGATDLNRKRTSLNENIYVKTYYGESHKTLA.GKQKADL

      290     300     310     320     330
rGehC NMF.LPFTITGNLICKAK.....EKWRENDGLVSVISSQHPFNQKYVERTD.KNQ
2HIH GME.FTKILTGNLICKAK.....EKWRENDGLVSVISSQHPFNQKYVERTD.KNQ
1KU0 GMNAFSAIVCAPFLGGRNAAALG...IDSHDLGNDGIVNTISMNGPKRGSDNRIVYDGT
5AH1 GPMNPIFYPTANILGRYSRNQKDLPIIDKKWFPPNDGVVNCISQDGPGLGSDNVIEQYNGG
4IIP GONDGVVSKSALYGVQLST.....SYKWNHDEINQLGVRGANAEDEVAVIRTHAN

      340     350     360     370     380
rGehC ..KGVWQVPTKHDWDHVDVFGQDSTDKRTRDLQGFHHGLAEGLVQSEQLTSTNK.
2HIH LHKGTWQVPTKHDWDHVDVFGQDSTDKRTRDLQGFHHGLAEGLVQSEQLTSTNK.
1KU0 LKKGTVWQVPTKHDWDHVDVFGQDSTDKRTRDLQGFHHGLAEGLVQSEQLTSTNK.
5AH1 VKLGQWNAFRIINTDHDIVGTFG...NVKDNVMDYASFLSLSLRALSHHHHHH..
4IIP RLKLSV.....

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Figure S1 Sequences of lipases were aligned using MultAlin. Visualization of the multiple sequence alignment was performed with ESPrpt 3.0. The consensus regions are colored based on sequence conservation: red, highly conserved (more than 90% sequence identity); yellow, moderately conserved (more than 50% sequence similarity); and white, not conserved. Amino acid sequences of *S. epidermidis* lipase rGehC, *S. hyicus* lipase (2HIH), *Bacillus stearothermophilus* lipase (1KU0), *Clostridium botulinum* esterase (5AH1) and *Pseudomonas cepacia* lipase (4IIP) α/β hydroxylase were obtained from the PDB.

Table S1 Kinetic parameters of recombinant rGehC

Substrate	(chain)	K_m (mM)	k_{cat} (s ⁻¹)	k_{cat}/K_m (s ⁻¹ mM ⁻¹)
<i>p</i> -NP butyrate	(C ₄)	1.03 ± 0.05	856.6 ± 34.6	836.6 ± 8.3
<i>p</i> -NP caprylate	(C ₈)	1.59 ± 0.24	680.6 ± 77.2	431.8 ± 14.1
<i>p</i> -NP decanoate	(C ₁₀)	1.02 ± 0.04	524.9 ± 24.4	637.3 ± 12.1
<i>p</i> -NP myristate	(C ₁₄)	0.52 ± 0.03	41.0 ± 3.3	79.2 ± 1.2

The activity was assayed in 50 mM Good's buffer (pH 6.0) with different substrate concentrations of substrates and 5 µg of protein at 37°C. Each data was expressed as the mean ± SE of three individual replication.