

A novel lipase from *Streptomyces exfoliatus* DSMZ 41693 for biotechnological applications

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Table S1. Strains, plasmids, and oligonucleotides used in this study.

Strain	Relevant description or sequence	Reference
<i>Streptomyces exfoliatus</i> DSMZ 41693	Producer of lipases	[26]
<i>Escherichia coli</i> DH5 α	Host for recombinant plasmids F ⁻ ϕ 80 <i>lacZ</i> Δ <i>M15</i> Δ(<i>lacZ</i> YA- <i>argF</i>)U169 <i>recA1</i> <i>endA1 hsdR17</i> (r _K ⁻ , m _K ⁺) <i>phoA supE44</i> λ ⁻ <i>thi-1</i> <i>gyrA96 relA1</i>	[101]
<i>Rhodococcus</i> sp. T104 KACC 21099	Host for gene expression (Kn ^s)	[102]
<i>Rhodococcus</i> sp. T104 KACC 21099 pENV19SeLipA	<i>Rhodococcus</i> sp. T104 KACC 21099 recombinant strain harboring the plasmid pENV19SeLipA	This study
<i>Rhodococcus</i> sp. T104 KACC 21099 pENV19SeLipB	<i>Rhodococcus</i> sp. T104 KACC 21099 recombinant strain harboring the plasmid pENV19SeLipB	This study
<i>Rhodococcus</i> sp. T104 KACC 21099 pENV19SeLipC	<i>Rhodococcus</i> sp. T104 KACC 21099 recombinant strain harboring the plasmid pENV19SeLipC	This study
<i>Rhodococcus</i> sp. T104 KACC 21099 pENV19SeLipD	<i>Rhodococcus</i> sp. T104 KACC 21099 recombinant strain harboring the plasmid pENV19SeLipD	This study
Plasmids		
pENV19	Shuttle vector for <i>E. coli</i> - <i>Rhodococcus</i> with the constitutive <i>perme</i> * promoter (5.1 Kb, Kn ^R pAL5000ori <i>perme</i> * ColE1ori)	[35,37,38]
pENV19SeLipA	pENV19 containing <i>lipA</i> gene from <i>S. exfoliatus</i> DSMZ 41693	This study
pENV19SeLipB	pENV19 containing <i>lipB</i> gene from <i>S. exfoliatus</i> DSMZ 41693	This study
pENV19SeLipC	pENV19 containing <i>lipC</i> gene from <i>S. exfoliatus</i> DSMZ 41693	This study
pENV19SeLipD	pENV19 containing <i>lipPs</i> gene from <i>S. exfoliatus</i> DSMZ 41693	This study
Primers		
Sequence (5'→3') [#]		
SeLipAFWsp	GCTCTAGAGAGGACGACTCCGTCATGAAACGACAC	This study
SeLipAREV	GGAATTCCTCAGGAACCGAGCGGGCAGGTG	This study
SeLipBFWsp	GCTCTAGAGGAGGTTCATGAGACTGTCC	This study
SeLipBREV	GGAATTCCTACGCCGCCCGTTG	This study
SeLipCFWsp	GCTCTAGACAGTCGTGGCTGGAACGACTGGTCCTGCAAGCCC	This study
SeLipCREV	GGAATTCCTAGCCGATCACCAGAGAGGCAGGTGGTCGGGGTCTG	This study
SeLipDFW	GCTCTAGAAGGAGTTCCTATGTCAGGGAG	This study
SeLipDREV	GGAATTCCTCAGGCCGTGGGACCGGGAATCTG	This study

[#]The restriction sites *Xba*I and *Eco*RI are shown in bold, ribosomal binding site sequence is shown in italics, and the start and the stop codons are shown underlined.

Table S2. PCR conditions for amplification of lipase genes from *Streptomyces exfoliatus* DSMZ 41693.

Step	Temperature (°C)	Time
1. Denaturalization	95	3 minutes
2. Denaturalization	95	30 seconds
3. Annealing	83	30 seconds
4. Extension	72	55 seconds
5. Repeat 17 cycles steps 2-4 decreasing 1 °C annealing temperature by cycle		
6. Denaturalization	95	30 seconds
7. Annealing	58-72	30 seconds
8. Extension	72	55 seconds
9. Repeat 20 cycles steps 6-8 increasing 1 s annealing time by cycle		
10. Extension	72	10 minutes

Table S3. pNP-ester hydrolytic specific activity in the culture broth of the recombinant strains.

pNP-esters (number of carbon atoms in acyl chain)	Specific activity (IU/mg protein)			
	SeLipA	SeLipB	SeLipC	SeLipD
pNP-acetate (C2)	n.d.	n.d.	n.d.	n.d.
pNP-butyrate (C4)	0.9	n.d.	0.9	n.d.
pNP-valerate (C5)	2.5	n.d.	2.5	n.d.
pNP-hexanoate (C6)	3.1	n.d.	6.5	n.d.
pNP-octanoate (C8)	4.8	n.d.	13.3	n.d.
pNP-decanoate (C10)	8.0	n.d.	17.2	n.d.
pNP-laurate (C12)	7.1	n.d.	12.8	n.d.
pNP-myristate (C14)	4.8	n.d.	9.5	n.d.
pNP-palmitate (C16)	5.3	n.d.	12	n.d.
pNP-stearate (C18)	4.6	n.d.	7.8	n.d.

n.d.: not detected

Table S4. Purification of SeLipC produced by *Rhodococcus* pENV19SeLipC by hydrophobic interaction chromatography (HIC).

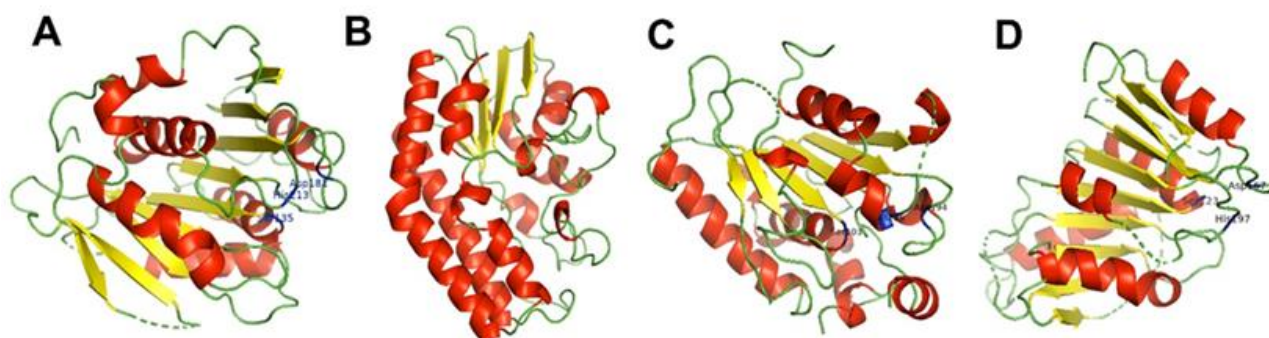
Step	V (mL)	Activity (IU)	Protein concentration (mg/mL)	Total protein (mg)	Specific activity (IU/mg)	Purification factor (fold)	Yield (%)
Broth	700	1356	0.163	114.1	12	1	100
Broth (NH ₄) ₂ SO ₄	740	1202	0.151	111.7	11	0.9	89
HIC	92	245.7	0.023	2.12	116.6	3.6	17.2
Dialysis	140	240.8	0.007	1.04	232.4	7.2	16.9

Table S5. Effect of different reducing agents on SeLipC activity.

Chemical reagent	Concentration	Residual activity (%)
No addition	-	100.0 ± 7.2
Dithiothreitol (DTT)	1 mM	94.9 ± 6.0
	10 mM	101.6 ± 5.7
2-Mercaptoethanol	1 mM	99.8 ± 1.9
	10 mM	99.4 ± 2.9

Table S6. Effect of chemical modification with PMSF on SeLipC.

Chemical reagent	Concentration	Residual activity (%)
<i>In absence of substrate (active site not protected)</i>		
No addition	-	100 ± 2.6
PMSF	1 mM	84.7 ± 3.5
	10 mM	51.2 ± 5.5
<i>In presence of substrate (active site protected)</i>		
No addition	-	100 ± 0.5
PMSF	1 mM	100.5 ± 1.2
	10 mM	90.0 ± 1.3

**Figure S1.** Predicted 3D structures of hypothetical lipases from *Streptomyces exfoliatus* DSMZ 41693. **A.** SeLipA; **B.** SeLipB; **C.** SeLipC; **D.** SeLipD. Models were obtained by RoseTTAFold program [29] and their structures were visualized by PyMOL program (Schrodinger, LLC, version 2.5).

gi|639917083 Mass: 31194 Score: 659 Expect: 1.3e-066 Matches: 21
lipase [*Streptomyces exfoliatus*]

Query	Start	End	Observed	Mr(expt)	Mr(calc)	ppm	M	Score	Peptide
59	84	109	2896.5251	2895.5178	2895.4418	26.2	0		R.GYCVFSLDYGQLPNVPFFHGLGPIAK.S
5	110	120	1266.5919	1265.5846	1265.5888	-3.28	0		K.SAEQLDAYVDR.V
6	110	120	1266.5919	1265.5846	1265.5888	-3.28	0	66	K.SAEQLDAYVDR.V
72	110	143	3544.8044	3543.7971	3543.6722	35.2	1		K.SAEQLDAYVDRVLAATGAPEADLVGHSQGGMMMPR.W + 2 Oxidation (M)
39	121	143	2297.1086	2296.1013	2296.0940	3.19	0		R.VLAATGAPEADLVGHSQGGMMMPR.W + 2 Oxidation (M)
56	121	147	2887.5613	2886.5540	2886.4157	47.9	1		R.VLAATGAPEADLVGHSQGGMMMPRWYLK.F + 2 Oxidation (M)
48	148	175	2836.6228	2835.6155	2835.5495	23.3	1		K.FLGGAKEVNTLVGIAPDNHGTLLGLTK.L
32	155	175	2134.1792	2133.1719	2133.1794	-3.50	0		K.VNTLVGIAPDNHGTLLGLTK.L
33	155	175	2134.1792	2133.1719	2133.1794	-3.50	0	69	K.VNTLVGIAPDNHGTLLGLTK.L
67	176	206	3163.7524	3162.7451	3162.6489	30.4	0		K.LLPYFPGAEDLISSATPGLADQIAGSAFITK.L
68	176	206	3163.7524	3162.7451	3162.6489	30.4	0	112	K.LLPYFPGAEDLISSATPGLADQIAGSAFITK.L
3	207	218	1213.6143	1212.6070	1212.6099	-2.35	0		K.LNEGSDTVPGVR.Y
4	207	218	1213.6143	1212.6070	1212.6099	-2.35	0	42	K.LNEGSDTVPGVR.Y
27	219	234	1939.9414	1938.9341	1938.9476	-6.94	0		R.YHVIASQYDEVVTPYR.S
28	219	234	1939.9414	1938.9341	1938.9476	-6.94	0	143	R.YHVIASQYDEVVTPYR.S
29	219	234	1939.9414	1938.9341	1938.9476	-6.94	0	152	R.YHVIASQYDEVVTPYR.S
22	235	251	1859.9799	1858.9726	1858.9789	-3.37	0		R.SQFLSGPNVTNLIQDK.C
12	252	267	1755.8586	1754.8513	1754.8621	-6.16	0		K.CALDSEHVAIGTVDR.V
70	252	281	3276.6643	3275.6570	3275.6357	6.51	1		K.CALDSEHVAIGTVDRVTFHEVANALDPAR.A
9	268	281	1539.7820	1538.7747	1538.7841	-6.12	0		R.VTFHEVANALDPAR.A
10	268	281	1539.7820	1538.7747	1538.7841	-6.12	0	70	R.VTFHEVANALDPAR.A

Figure S2. Peptide mass fingerprinting of SeLipC obtained by MALDI-TOF analysis. The protein was identified as a lipase from *Streptomyces exfoliatus* (gi|639917083) after peptide matching process using MASCOT Software (Score 659).

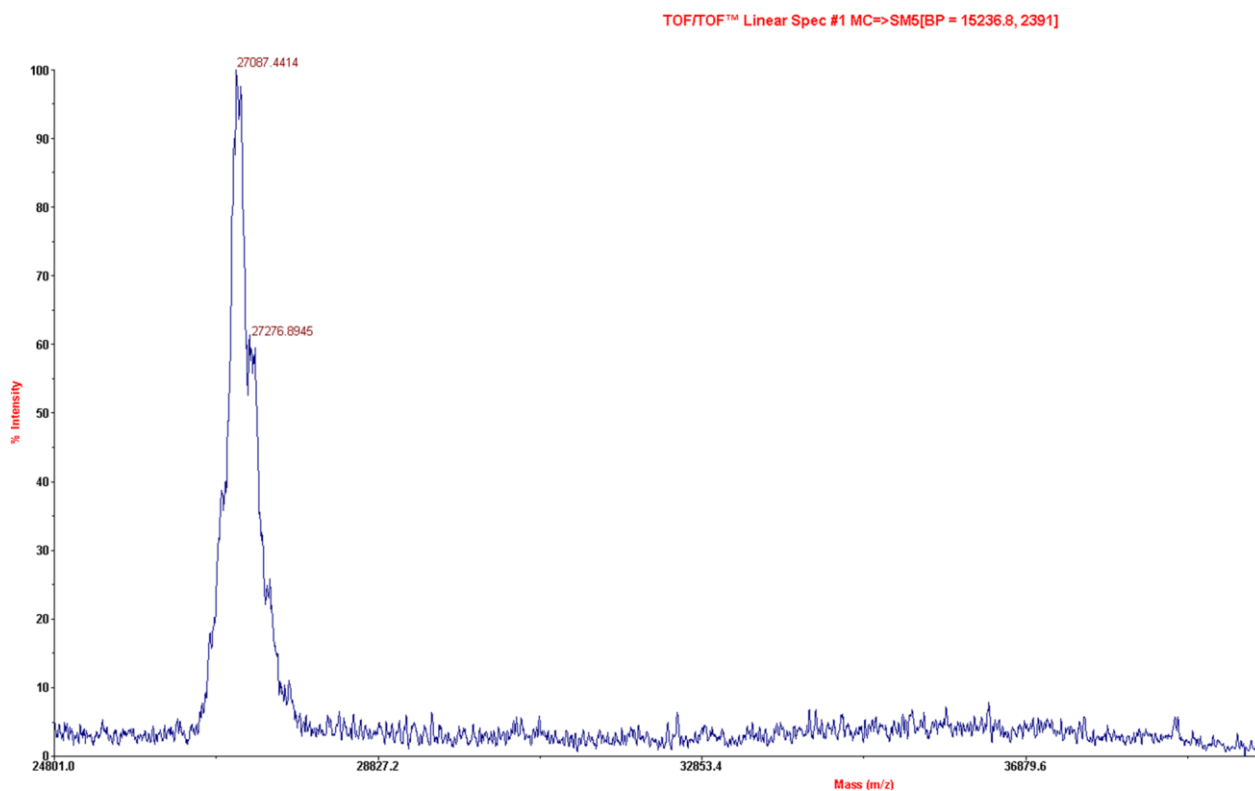


Figure S3. Determination of the molecular mass of SeLipC by MALDI-TOF. Mass spectrum recorded with 0.127 mg/mL of pure lipase.

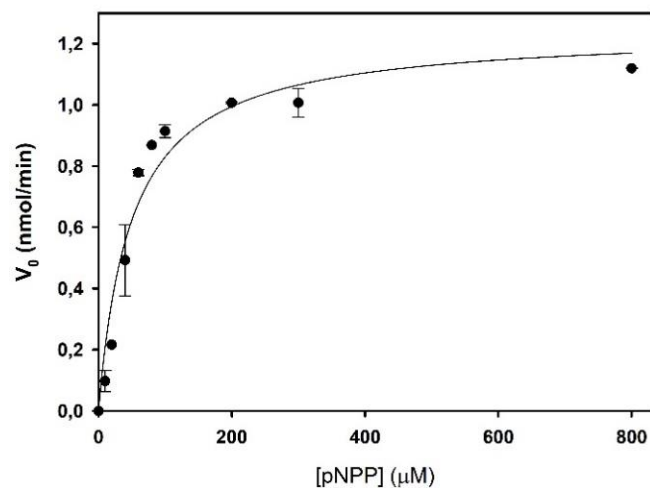


Figure S4. Michaelis-Menten hyperbola plot for the determination of kinetic parameters of SeLipC using pNPP as substrate. Conditions: 200 μL assay volume, 0.05 $\mu\text{g/mL}$ of enzyme (1.81 nM), assay time 10 min, 37°C, 50 mM Tris HCl pH 8.0, 0.01% gum arabic and 0.02% sodium deoxycholate. $K_M = 49.6 \pm 11.2 \mu\text{M}$ and $V_{max} = 1.24 \pm 0.08 \times 10^{-3} \mu\text{moles/min}$ ($k_{cat} = 57 \text{ s}^{-1}$ and $k_{cat} / K_M = 1.15 \times 10^6 \text{ s}^{-1} \cdot \text{M}^{-1}$).

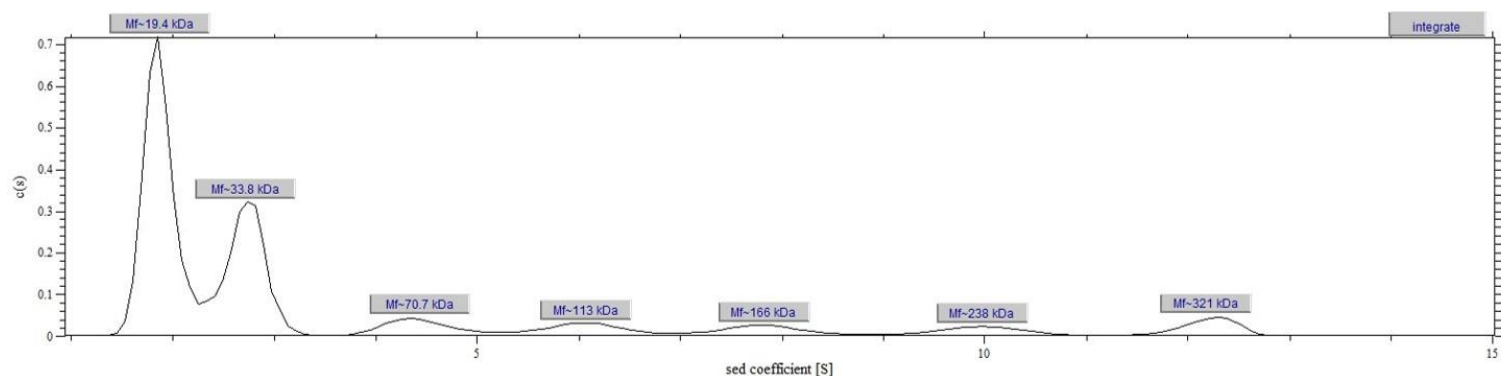


Figure S5. Analytical ultracentrifugation analysis of SeLipC: $c(s)$ distribution as a function of sedimentation coefficient (S). Experiment was performed with 0.320 mg/mL of pure lipase, and the presence of two main species with sedimentation coefficient (S) values of 1.9 and 2.7 were observed. These values are compatible with the presence of monomers and dimers with spheroid oblate-shape in both forms.