

Supplementary material

1. Codon-optimized gene sequence of *Rhizopus oryzae* lipase (GenBank: GQ502721.1) based on

Pichia pastoris was listed below:

```
atggtttcattcatttccatttctcaaggtgtagctttgtcttctgtcttccatgatgctcggttcatctgctgttctgttctggtaaacttgatct
tccactaccgccgtctctgcatctgacaattctgccctccctctctcatttccagccgttgctcctccttaacaagggaaagtaaagcgatc
ttcaagctgaaccttactacatgcaaagaatacagaatggatgagctccatgggtggcaacctgacatccatcggaaagcgagatgacaattt
ggttggtggcatgacttggattacctagcggatgctcctctatcagcctctctggatctaccaacagcgcctctgatgggtgtaagggtgctgct
gctactactgctcaaattcaagagttcaccaagtagctggatcgctgccactgcctactgtcgttctgttgcctggtaacaagtgaggactgtg
tccaatgtcaaaagtggttctctgatggcaagatcatcactacctttacctccttgccttccgacacaaatggttacgtcttgagaagtataaaca
aaagaccatttatcttgtttccgtgggtaccaactcctcagaagtgccatcactgatattgttcaactttccgactacaagcctgtcaagggcg
ccaaggtcatgctggttcccttctcttatgagcaagttgcaatgactattccctgtcatccaagaacaactgaccgtaaccctactfacaag
gtcatcgttaccggctactcactcgggtgggcacaagctttgcttccggatggatctctaccaacgtgaaccaagattgtctccaagaatttg
agcatcttactgttgggtgctcctgtgttgtaacccaccttcttactatgttgaatctaccggtattccttccaacgtaccgttcacaagaga
gatategttcctcacgttctcctcaatcctcggattccttcaccccggtgttgaatcttggatcaagtccggctacctccaacgttcaaatctgact
tctgaaattgaaaccaaggattgcagtaactctatcgttcccttcacctctccttgatcacttgagttactttgatatcaacgaaggaagctgtttgt
aa
```

2. **Amino acid sequence of *Rhizopus oryzae* lipase.**

The entire protein contains 392 amino acid residues[1]. From the first amino acid residue to the 26th is the signal peptide, followed by a 96 amino acid residue leader peptide. The mature peptide starts from the 124th amino acid residue and has a total of 269 amino acid residues. Gene cloning begins from the leader peptide, but the amino acid residual numbering in the article begins from the mature peptide. Amino acid sequence of *Rhizopus oryzae* lipase was shown in figure S1:



Figure S1. Amino acid sequence of *Rhizopus oryzae* lipase

3. Results of disulfide bond prediction by DbD2

Table S1. Results of disulfide bond prediction by DbD2

Predicted Disulfide Bond	Original Residues	Score	Energy	Bad Contacts	Thermal Mobility	Sequence Separation	Residue Depth	Volume Change	Environment	Chi3
CYS106:SG - CYS185:SG	A:ALA106, A:PHE185	97.7	1.15	0	32.51	79	5.91	-75.6	Coil-Helix	102.1
CYS112:SG - CYS149:SG	A:PHE112, A:ALA149	97.63	1.19	0	26.87	37	6.64	-75.6	Helix-Helix	-115.7
CYS190:SG - CYS238:SG	A:GLU190, A:GLU238	95.82	2.09	0	38.48	48	3.78	-76.6	Coil-Helix	-95.3
CYS203:SG - CYS253:SG	A:ARG203, A:SER253	94.49	2.75	0	45.27	50	4.38	-82.7	Coil-Turn	102.1
CYS186:SG - CYS217:SG	A:ALA186, A:LEU217	93.9	3.05	0	24.77	31	4.99	-46.9	Coil-Helix	104.8
CYS196:SG - CYS220:SG	A:PHE196, A:GLY220	92.95	3.52	0	31.19	24	5.77	-49.9	Coil-Sheet	85.2
CYS20:SG - CYS173:SG	A:TYR20, A:PHE173	91.57	4.21	0	26.41	153	8.22	-182.6	Helix-Sheet	-122.2
CYS49:SG - CYS72:SG	A:ASP49, A:LYS72	91.09	4.46	0	42.54	23	3.42	-80.4	Turn-Turn	160.6
CYS141:SG - CYS151:SG	A:VAL141, A:ALA151	90.36	4.82	0	21.44	10	10.7	-21.5	Helix-Sheet	169.1
CYS54:SG - CYS67:SG	A:THR54, A:VAL67	89.61	5.2	0	26.85	13	5.7	-52.2	Sheet-Sheet	148.3
CYS210:SG - CYS216:SG	A:PRO210, A:PHE216	89.6	5.2	0	22.23	6	5.65	-109.1	Coil-Coil	-110
CYS200:SG - CYS207:SG	A:VAL200, A:PRO207	88.12	5.94	0	33.03	7	8.93	-55	Helix-Sheet	-110.5
CYS33:SG - CYS55:SG	A:VAL33, A:THR55	87.8	6.1	0	28.84	22	5.27	-52.2	Sheet-Turn	-77.1
CYS105:SG - CYS184:SG	A:GLY105, A:THR184	86.18	6.91	0	28.97	79	3.11	22.5	Helix-Turn	53.7
CYS81:SG - CYS115:SG	A:SER81, A:SER115	86.07	6.97	0	32.69	54	3.83	17.5	Helix-Turn	80.8
CYS56:SG - CYS122:SG	A:PHE56, A:ASP122	85.75	7.13	0	28.21	66	3.52	-103.5	Helix-Sheet	-86.1
CYS26:SG - CYS42:SG	A:THR26, A:GLN42	85.67	7.16	0	28.59	16	6.95	-63.6	Helix-Helix	168.6
CYS65:SG - CYS80:SG	A:GLY65, A:PHE80	85.29	7.35	0	24.71	15	9.81	-49.9	Sheet-Sheet	109.8
CYS27:SG - CYS81:SG	A:ALA27, A:ARG81	85.26	7.37	0	25.42	54	8.49	-77.8	Sheet-Turn	-85.9
CYS224:SG - CYS245:SG	A:TRP224, A:SER245	84.98	7.51	0	39.1	21	7.5	-118.1	Helix-Sheet	-141.6
CYS58:SG - CYS63:SG	A:SER58, A:THR63	84.51	7.75	0	23.95	5	5.3	-8.1	Sheet-Sheet	151
CYS145:SG - CYS176:SG	A:SER145, A:GLY176	83.76	8.12	0	25.12	31	10.74	48.2	Coil-Turn	106.3
CYS24:SG - CYS144:SG	A:ALA24, A:HIS144	83.72	8.14	0	18.98	120	11.7	-41.9	Helix-Sheet	-161.2
CYS108:SG - CYS181:SG	A:VAL108, A:GLY181	83.09	8.46	0	24.4	73	7.66	4.1	Coil-Sheet	83.1
CYS98:SG - CYS110:SG	A:SER98, A:ALA110	80.96	9.52	0	34.58	12	3.49	22.5	Helix-Sheet	118.7
CYS107:SG - CYS182:SG	A:LYS107, A:ASN182	80.08	9.96	0	27.94	75	4.7	-88.8	Coil-Sheet	164.7
CYS204:SG - CYS256:SG	A:ASP204, A:ASP256	66.63	6.68	1	40.05	52	6.07	-28.4	Coil-Helix	-78.1

4. Primers

Table S2. Primers used in this work

Mutants	Primers
Q15N	Farward:5'ACTACTGCTCAAATTAACGAGTTTACCAAG3'
	Reverse:5'CTTGGTAAACTCGTTAATTTGAGCAGTAGT3'
A24S	Farward:5'AAGTACGCTGGTATTTCTGCAACTGCTTAT3'
	Reverse:5'ATAAGCAGTTGCAGAAATACCAGCGTACTT3'
V41I	Farward:5'AACAAATGGGATTGTATCCAGTGTGAGAAG3'
	Reverse:5'CTTCTGACACTGGATAACAATCCCATTTGTT3'
L68A	Farward:5'ACAAACGGTTACGTCGCTAGAAGTGATAAA3'
	Reverse:5'TTTATCACTTCTAGCGACGTAACCGTTTGT3'
A110D	Farward:5'GGTGCCAAAGTGCATGATGGATTCCCTAAGT3'
	Reverse:5'ACTTAGGAATCCATCATGCACTTTGGCACC3'
Y123L	Farward:5'CAAGTCGTGAACGATTTGTTCCAGTGGTT3'
	Reverse:5'AACCACTGGGAACAAATCGTTCACGACTTG3'
E129D	Farward:5'TTCCCAGTGGTTCAAGATCAATTGACGGCC3'
	Reverse:5'GGCCGTCAATTGATCTTGAACCACTGGGAA3'
T184A	Farward:5'AGAGTTGGCAATCCCGCCTTTGCCTATTAT3'
	Reverse:5'ATAATAGGCAAAGGCGGGATTGCCAACTCT3'
V209L	Farward:5'GACATTGTTCCACACCTACCACCACAATCA3'
	Reverse:5'TGATTGTGGTGGTAGGTGTGGAACAATGTC3'
S223Y	Farward:5'CACCCCGGCGTTGAATATTGGATTAAGTCC3'
	Reverse:5'GGACTTAATCCAATATTCAACGCCGGGGTG3'
D262G	Farward:5'CACTTATCCTACTTTGGGATTAATGAGGGG3'
	Reverse:5'CCCCTCATTAAATCCCAAAGTAGGATAAGTG3'
A106C	Farward:5'AAACCCGTCAAAGTTGTAAAGTGCATGCCGGA3'
	Reverse:5'TCCGGCATGCACTTTACAACCTTTGACGGGTTT3'
F185C	Farward:5'GTTGGCAATCCCACATGTGCCTATTATGTTGAA3'
	Reverse:5'TTCAACATAATAGGCACATGTGGGATTGCCAAC3'
F112C	Farward:5'AAAGTGCATGCCGGATGTCTAAGTTCTTATGAA3'
	Reverse:5'TTCATAAGAACTTAGACATCCGGCATGCACTTT3'
A149C	Farward:5'CATAGTTTGGGTGGATGTCAAGCCTTACTAGCC3'
	Reverse:5'GGCTAGTAAGGCTTGACATCCACCCAACTATG3'
E190C	Farward:5'TTTGCCTATTATGTTTGTCCACTGGCATAACCA3'
	Reverse:5'TGGTATGCCAGTGAACAACATAAATAGGCAAA3'
E238C	Farward:5'CAAATTTGTACTTCCTGTATTGAAACCAAAGAC3'
	Reverse:5'GTCTTTGGTTTCAATACAGGAAGTACAAATTTG3'
R203C	Farward:5'AGGACTGTTCAAGTGTGACATTGTTCCACAC3'
	Reverse:5'GTGTGGAACAATGTCACACTTGTGAACAGTCCT3'

S253C	Farward:5'ATAGTCCCCTTCACCTGTTTATTAGACCACTTA3' Reverse:5'TAAGTGGTCTAATAAACAGGTGAAGGGGACTAT3'
A186C	Farward:5'GGCAATCCCACATTTTGTATTATGTTGAATCC3' Reverse:5'GGATTCAACATAATAACAAAATGTGGGATTGCC3'
L217C	Farward:5'CAATCATTTGGGTTTTGTACACCCCGGCGTTGAA3' Reverse:5'TTCAACGCCGGGGTGACAAAACCCAAATGATTG3'
F196C	Farward:5'TCCACTGGCATAACCATGTCAAAGGACTGTTAC3' Reverse:5'GTGAACAGTCCTTTGACATGGTATGCCAGTGGAA3'
G220C	Farward:5'GGGTTTCTACACCCCTGTGTTGAATCTTGGATT3' Reverse:5'AATCCAAGATTCAACACAGGGGTGTAGAAACCC3'
Y20C	Farward:5'CAGGAGTTTACCAAGTGTGCTGGTATTGCTGCA3' Reverse:5'TGCAGCAATACCAGCACACTTGGTAAACTCCTG3'
F173C	Farward:5'AAGAACTTGTC AATATGTACGGTCGGAGGACCA3' Reverse:5'TGGTCCTCCGACCGTACATATTGACAAGTTCTT3'
D49C	Farward:5'CAGAAGTGGGTTCTTGTGGAAAGATCATCACA3' Reverse:5'TGTGATGATCTTTCCACAAGGAACCCACTTCTG3'
K72C	Farward:5'GTCTTGAGAAGTGATTGTCAGAAGACCATCTAC3' Reverse:5'GTAGATGGTCTTCTGACAATCACTTCTCAAGAC3'
V141C	Farward:5'ACTTACAAAGTAATATGTACCGGTCATAGTTTG3' Reverse:5'CAA ACTATGACCGGTACATATTACTTTGTAAGT3'
A151	Farward:5'TTGGGTGGAGCACAATGTTTACTAGCCGGAATG3' Reverse:5'CATTCCGGCTAGTAAACATTGTGCTCCACCCAA3'

Reference:

- [1] M. Ueda, S. Takahashi, M. Washida, S. Shiraga, A. Tanaka, Expression of *Rhizopus oryzae* lipase gene in *Saccharomyces cerevisiae*, *Journal of Molecular Catalysis B Enzymatic* 17(3) (2002) 113-124.