*molecules*

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*Supplementary Materials*

# Cold-active lipase from the ice cave *Psychrobacter* SC65A.3 strain, promising biocatalyst for silybin acylation

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## *Psychrobacter* SC65A3.1 *lip*2 gene [OQ547793.1]

ATGTCTAACTCAACAGTACTATCAGTCAACACCTTACTCAATAAAGCGGTGAA-
GACCCTAAATCTTATGTCATTTGGACAAGATAAGAACCCCAAAAGCACTGATATCAATTTATCAGATGAAATAATAGATATAGAA
GAGAGTGCTTTGCAAGATAGTCGCGAGGATAAAGGCTTATCTATTAAAGAAAAGATACTT-
GAGCACCATCTGATGACCAACTATCAACCGCATCTACTGCACTATGCCATAAAAAGCTTTGGCTGCTTACCTACGCCGATTCTTGA
GAGTTTGATAAAGTGTTTAGATGGACCTACTTCAAAGCAATATTTGCATGTT-
GATGCCCATCTGCGCTTAATACTTGCGGTCAATAGCAAGCTAAAAACGCCTTTGCAGCTGATAGAGATGTCTGAGCTGCGTAAAC
GCTTTGCAACAGATGCGGTCGCTATGCAAGCACCAAAAGTATGGCAGCAA-
GCTTCTGACAACTTGCTTAGCAATTTGAAACAGTTTCATAAAAAAGGCGATAGCGCTATCAGTTGGCAAGATAGAACGATTACCA
ATGCCGACGACGGTGATATGACCATTCGCTGCTATCAAAATGAGACCTCTGACAATGGTTTT-
GGCTTTAAAAAAGAGCAAACCAGTAATCCTGATGAGACCGTGCTATTGTTTTTTCATGGTGGTGGATTTTGTATTGGTGACCTTA
ATACCCATCATGAATTTTGTCATGCCATTTGTGAGCAAACGGGCTGGCCGGTCATTAGTGTT-
GATTATCGCTTAGCACCTGAACACCCTGCCCCTGCTGCCGTAAGAGACTGTATCAGTGCCTATGCTTGGTTAGCTGAACATTGTGA
AGAATTTGGTGCTTTACCATCACGCATTGTATTAGCAGGTGATAGCGCAGGTGGTGGAT-
TATCTACTTTGATGGCTCAGCAAATCATCACGCCAAATAAAGAGGCTTGGTTGGATCTAGGTGATGAAGGTCAAAAGACCTTTGA
TATATTACAAGGTTTACCACATCCTATGGCACAGATGCCCTTATATCCTGTGACCGA-
TATTGAGACCGATTATCCAAGCTGGGAGTTATATGGTGAAGGTCTATTACTCGACCATGCTGATGTTGCCATTTTTGATGCCGCTT
GTTTAGAAAATAGTCCGTTACCGCGCCAGCATATCCTGACCTCACCGATGCTTGGG-
GACAATCGACAGGTTTGTCCAAGTTATGTCGTTGCAGCAGAATTAGATGTCTTACGTGATGAAGCGTTTGCTTATGCCAATCAGC
TAAAGAGTTTTGGTATAGCCGTACAAACCCATACGGTACTTGGCGCACCGCATGGAT-
TTATTCACTTTATGAGCGTTCATCAAAGATTAGGGCAAGAAACTCAGCATATCATCACAGGGTTTGCTAATTTTGTGCGTGAAATC
ATAAAAACAAGGGCGCTATTGAGCGCTTAA

## PSL2 Lipase [WP_263241157.1]

MSNSTVLSVNTLLNKAVKTLNLMSFGQDKNPKSTDINLSDEIIDIEESALQDSREDKGL-
SIKEKILEHHLMTNYQPHLLHYAIKSFGCLPTPILESLIKCLDGPTSKQYLHVDAHLRLILAVNSKLKTPLQLIEMSELRKRFATDAVAMQA
PKVWQQASDNLLSNLKQFHKKGDSAISWQDRTITNADDGDMTIR-
CYQNETSDNGFGFKKEQTSNPDETVLLFFHGGGFCIGDLNTHHEFCHAICEQTGWPVISVDYRLAPEHPAPAAVRDCISAYAWLAEHC
EEFGALPSRIVLAGDSAGGGLSTLMAQQIITPNKEAWLDLGDEGQKTFDILQGLPHP-
MAQMPLYPVTDIETDYPSWELYGEGLLLDHADVAIFDAACLENSPLPRQHILTSPMLGDNRQVCPSYVVAAELDVLRDEAFAYANQLK
SFGIAVQTHTVLGAPHGFIHFMSVHQRLGQETQHIITGFANFVREIIKTRALLSA

**Figure S1.** *Psychrobacter* SC65A3.1 *lip2* gene nucleotide sequence cloned in pHAT2 vector and corresponding amino acid sequence of PSL2 lipase. (Underlined sequence): cloning restriction sites *Nco*I (CCATGG) and *Bam*HI (GGATCC) in pHAT2 vector; (Bold) start and stop codons of the *lip*2 gene .

**Table S1.** Amino acid composition of PSL2.

| Amino acid | Number | Content |
|---|---|---|
| Ala (A) | 40 | 8.3% |
| Arg (R) | 15 | 3.1% |
| Asn (N) | 20 | 4.1% |
| Asp (D) | 32 | 6.6% |
| Cys (C) | 10 | 2.1% |
| Gln (Q) | 25 | 5.2% |
| Glu (E) | 28 | 5.8% |
| Gly (G) | 29 | 6.0% |
| His (H) | 21 | 4.3% |
| Ile (I) | 31 | 6.4% |
| Leu (L) | 56 | 11.6% |
| Lys (K) | 23 | 4.8% |
| Met (M) | 11 | 2.3% |
| Phe (F) | 19 | 3.9% |
| Pro (P) | 24 | 5.0% |
| Ser (S) | 32 | 6.6% |
| Thr (T) | 28 | 5.8% |
| Trp (W) | 6 | 1.2% |
| Tyr (Y) | 11 | 2.3% |
| Val (V) | 22 | 4.6% |
| Pyl (O) | 0 | 0.0% |
| Sec (U) | 0 | 0.0% |
| (B) | 0 | 0.0% |
| (Z) | 0 | 0.0% |
| (X) | 0 | 0.0% |

**Total number of negatively charged residues (Asp + Glu):** 60
**Total number of positively charged residues (Arg + Lys):** 38

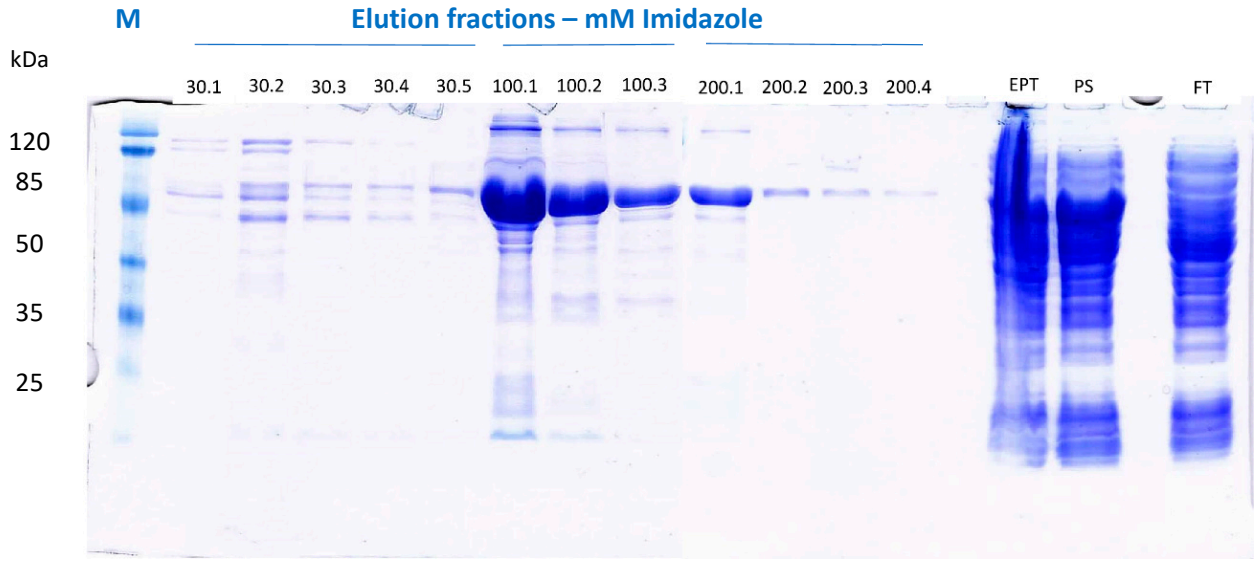



**Figure S2.** Purification of the recombinant PSL2 by affinity chromatography on Ni-NTA column. The purification fractions analyzed by SDS-PAGE (M): standard molecular mass proteins (Thermo Scientific); (30.1-30.3, 100.1-100.3, 200.1-200.4) fractions containing various concentrations of imidazole (30 mM, 100 mM, 200 mM) in TN buffer (100 mM TrisHCl, pH 8, 200 mM NaCl). (EPS): total extract; (PS): soluble extract; (FT): flow through.