S-oph enzyme for efficient degradation of Polyvinyl alcohol: Soluble expression and Catalytic properties

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Table S1 Plasmid and molecular chaperones used in this study.

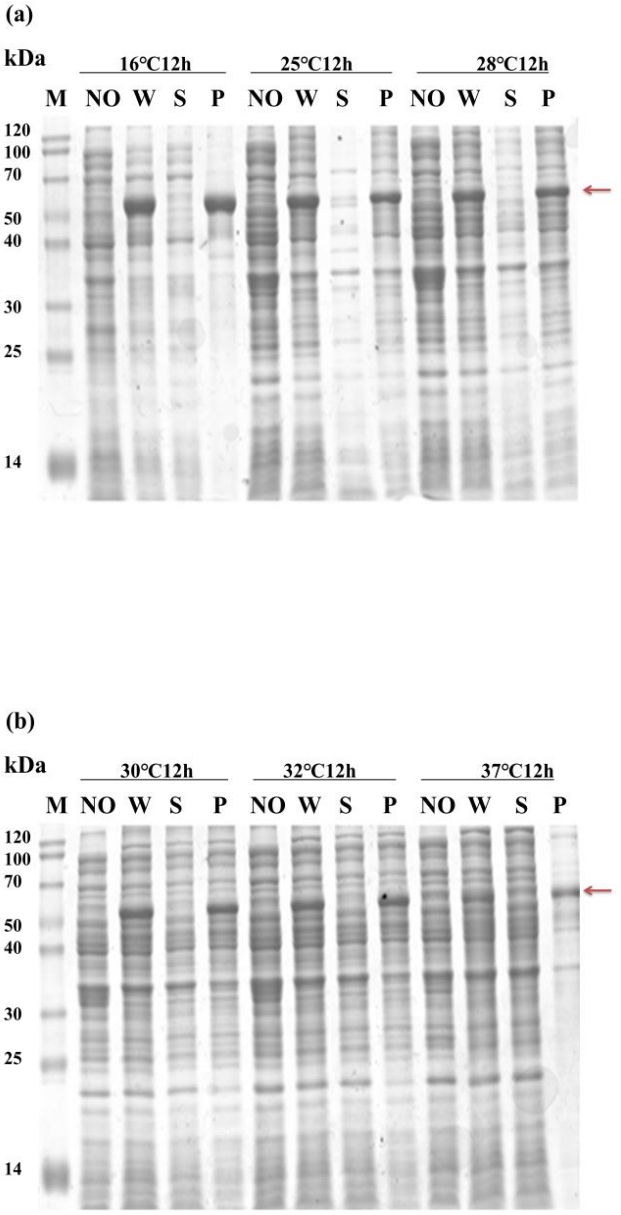
Plasmids	Description	Source
pET32a	*E. coli* expression vector	Addgene
pGro7	molecular chaperone	Takara Bio (Beijing)
pKJE7	molecular chaperone	Takara Bio (Beijing)
pTF16	molecular chaperone	Takara Bio (Beijing)

Table S2 Double digestion and seamless cloning system.

Double digestion (50 μL)		Seamless cloning (5 μL)	
10 × Single Buffer	5 μL	2 × Seamless cloning Master Mix	2.5 μL
*Not*I & *Sac*I	5 μL	Vector	2.5 μL
pET32a	5 μL	Fragment	2.5 μL
ddH_2O	5 μL		
37℃ 1 h		50℃ 20 min	

Table S3 All primers used in this study.

Named	Sequence (5'-3')
F	TATGGGAGAGAGGCATGTTCAAACCAGTTGTCAAGTCCCGTT
R	ATCGCTTCTGTGAGCTTGCCTTATAGTGATCAGTAAAACGACCAATC
s-oph-F	TATCGGATCCGAATTCGAGCTCATGTTCAAACCAGTTGTCAAGTC
s-oph-R	TGGTGGTGCTCGAGTGCGGCCGCTTACTTATAGTGATCAGTAAAACGACC
T7	TAATACGACTCACTATAGG
T7T	CCGCTGAGCAATAACTAGCA
1-6 × His-tag (s-oph)-F	TATCGGATCCGAATTCGAGCTCATGTTCAAACCAGTTGTCAAGTC
1-6 × His-tag (s-oph)-R	TGGTGGTGCTCGAGTGCGGCCGCCTTATAGTGATCAGTAAAACGACC
1-6 × His-tag (pET32a)-F	GTTTTACTGATCACTATAAGGCGGCCGCACTCGAGCACCACCACCA
1-6 × His-tag (pET32a)-R	TTGACAACTGGTTTGAACATGAGCTCGAATTCGGATCCGATATCAGC
3-6 × His-tag (s-oph)-F	TATCGGATCCGAATTCGAGCTCATGTTCAAACCAGTTGTCAAGTC
3-6 × His-tag (s-oph)-R	GCAGCCGGATCTCAGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGCTTATAGTGATCAGTAAAACG
3-6 × His-tag (pET32a)-F	CACCACCACCACTGAGATCCGGC
3-6 × His-tag (pET32a)-R	GAGCTCGAATTCGGATCCGATATCAGC


(a)
kDa
16°C12h
25°C12h
28°C12h
M NO W S P NO W S P NO W S P
120
100
70
50
40
30
25
14
(b)
kDa
30°C12h
32°C12h
37°C12h
M NO W S P NO W S P NO W S P
120
100
70
50
40
30
25
14

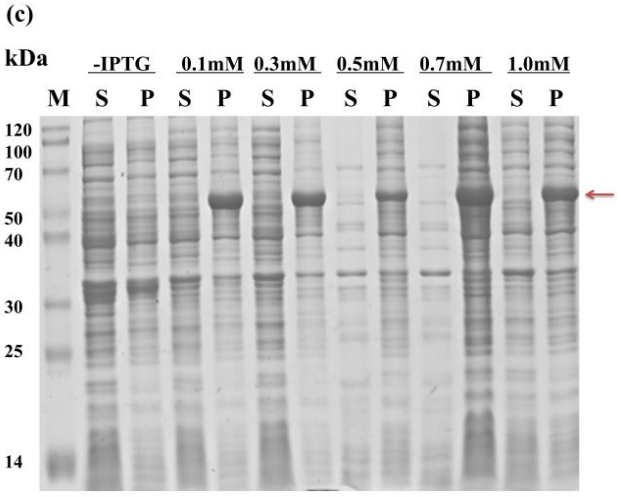


Figure S1 Expression of recombinant pET32a/s-oph/BL21 (DE3) strain under different temperature and different gradient concentrations of inducer. M: marker; NO: The cells were not induced with IPTG; W: The whole lysate; S: The supernatant fractions of cells after sonication; P: The insoluble pellets of cells after sonication; (a)/(b) pET32a/s-oph/BL21 (DE3) expression induced by different temperature gradients (c) pET32a/s-oph/BL21 (DE3) expression induced by different IPTG gradients

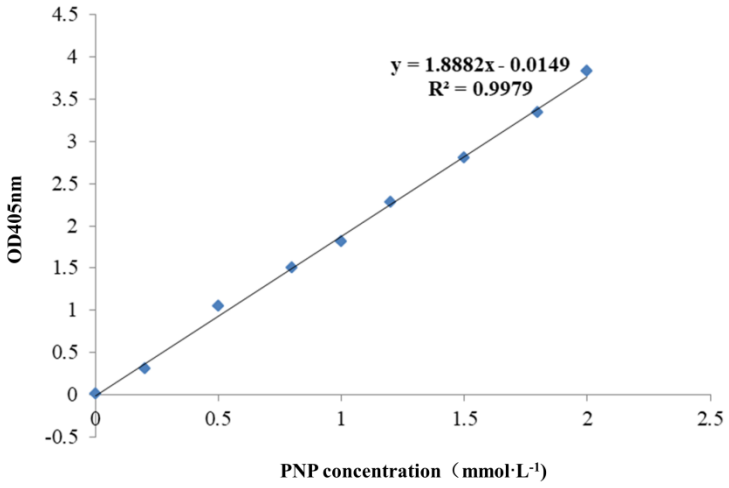


Figure S2 Standard curves of p-nitrophenol concentration and OD at 405 nm.