

1 **Simultaneous cell disruption and semi-quantitative activity assays for high-**  
2 **throughput screening of thermostable L-asparaginases**

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18 **Supplementary data**

19 **Supplementary table 1. The optimized sequence of *P. yanosii* CH1 L- asparaginase gene**

20 **for *Bacillus subtilis*.**

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sequences

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atgagactgctgatcctgggaatgggaggaacaatcgcaagtgtgccttcagaagaggatacgaatcatcactgtctgtggaggagatc  
ctgagactgcaggacttgagctgaagtgggaagttgaggctagagatctgctgaacatcgattctacgttgatccagcctgaggattgggt  
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cgcatgtcggggaagtccggcgcttaatgtaaccaatatggtcggcgaaattgggaaatccgcgtaa

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23 **Supplementary table 2. The character of L-asparaginases from different sources.** NR, not  
 24 reported. The  $V_{\max}$  of *Pectobacterium carotovorum* MTCC 1428 L-Asnase was 4.45U/ug.

Strains	GenBank accession No.	Specific activity (U/mg)	Optimum temperature (°C)	Optimum pH	$V_{\max}$ ( uM/min )	Km (mM)	Reference
<i>Pyrococcus yayanosii</i> CH1	WP_013906452.1	1483	95	8	2929	6.5	This study
<i>E.coli</i>	WP_000394140.1	105.2	37	7.5	NR	0.03	Maggi et al., 2017
<i>B. subtilis</i> B11- 06	AGT62618.1	92.45	40	7.5	77.51	0.43	Jia et al., 2013
<i>T.gammatolerans</i> EJ3	WP_015859055.1	7622	85	8.5	NR	10	Zuo et al., 2014
<i>T. zilligii</i> AN1 TziAN1_1	WP_010478656.1	5526	90	8.5	NR	6.02	Zuo et al., 2015
<i>Thermococcus kodakaraensis</i> KOD1	WP_011250607.1	2350	85	9.5	3300	5.5	Chohan & Rashid, 2013
<i>Pyrococcus furius</i> DSM 3638	WP_011013191.1	550	80	9	NR	12	Bansal et al., 2010
<i>Pectobacterium carotovorum</i> MTCC 1428	AFA36648.1	153.25	30	6.5		0.657	Kumar et al., 2011
<i>vibrio succinogenes</i>	WP_011138590.1	202	37	7.3	NR	0.478	Distasio et al., 1976

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26 **Supplementary table 3. The percentage of secondary structure in L-Asnase and its mutations.**

27 This results were calculated by Online server DICHROWEB.

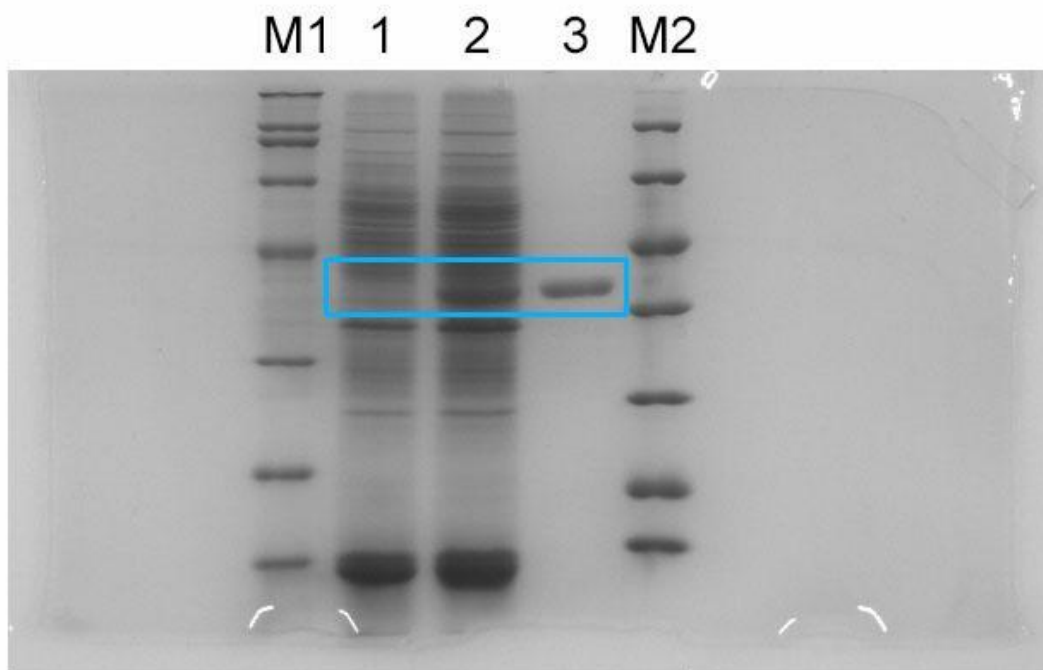
	$\alpha$ -helix(%)	$\beta$ -sheet(%)	$\beta$ -turn(%)	random coil(%)
L-Asnase	42.3	15.4	17.2	26.0
S17G	42.1	15.3	17.4	25.8
A90S	42.3	15.2	17.0	26.1
R156S	42.2	15.1	17.1	26.2
K272A	42.1	15.5	17.2	25.9

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30 **Supplementary table 4. The orthogonal experiments.**

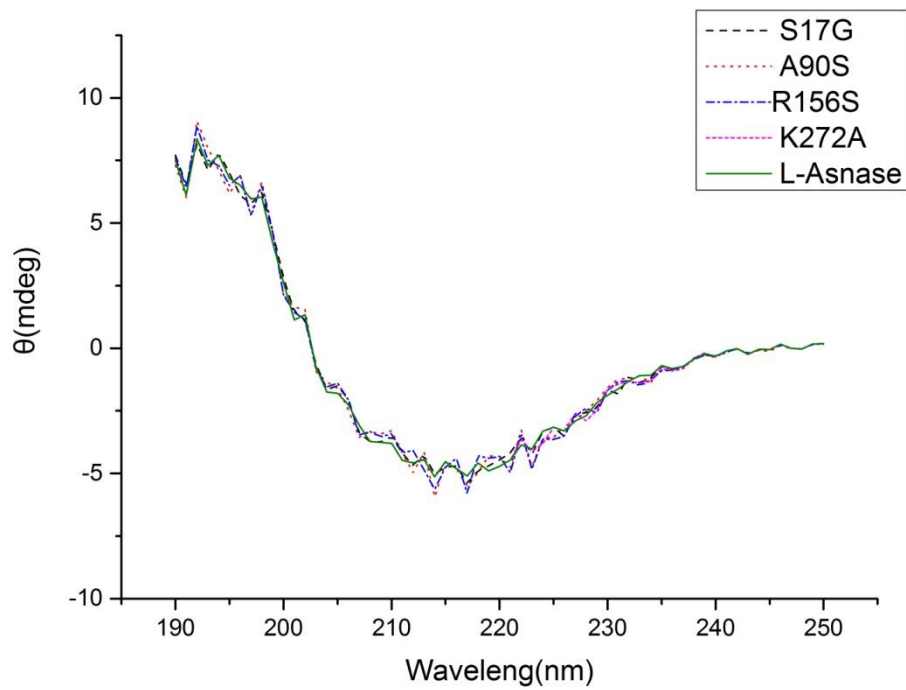
Factor	yeast (g/L)	glycerin (g/L)	NH <sub>4</sub> Cl(g/L)	corn steep liquor (g/L)	activity (U/mL)
Test 1	20	37	1	12	114.88
Test 2	20	42	1.5	15	128.7
Test 3	20	47	2	18	98.45
Test 4	20	52	2.5	21	86.3
Test 5	25	37	1.5	18	105.3
Test 6	25	42	1	21	90.2
Test7	25	47	2	12	134.95
Test 8	25	52	2.5	15	129.45
Test 9	30	37	2	21	96.3
Test 10	30	42	2.5	18	107.5
Test 11	30	47	1	15	149.67
Test 12	30	52	1.5	12	141.18
Test 13	35	37	2.5	15	134.18
Test 14	35	42	2	12	138
Test 15	35	47	1.5	21	108.3
Test 16	35	52	1	18	119.2
average value					
1	107.08	112.66	118.48	131.10	
average value					
2	114.98	116.10	120.87	136.65	
average value					
3	123.66	121.70	117.93	107.62	
average value					
4	124.93	120.18	113.35	95.28	
range	17.85	9.04	7.52	35.82	



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32 **Supplementary figure 1. SDS-PAGE analysis of L-asparaginase from *B. subtilis* 168/pMA5-**  
33 ***pyasnase*.** Lane M1, Premixed Protein Marker(Broad); Lane 1, *B. subtilis* 168/pMA5 cell extract; Lane 2,  
34 crude enzyme of *B. subtilis* 168/pMA5- *pyasnase*; Lane 3, the purified L- asparaginase; Lane M2,  
35 Unstained Protein Molecular Weight Marker.

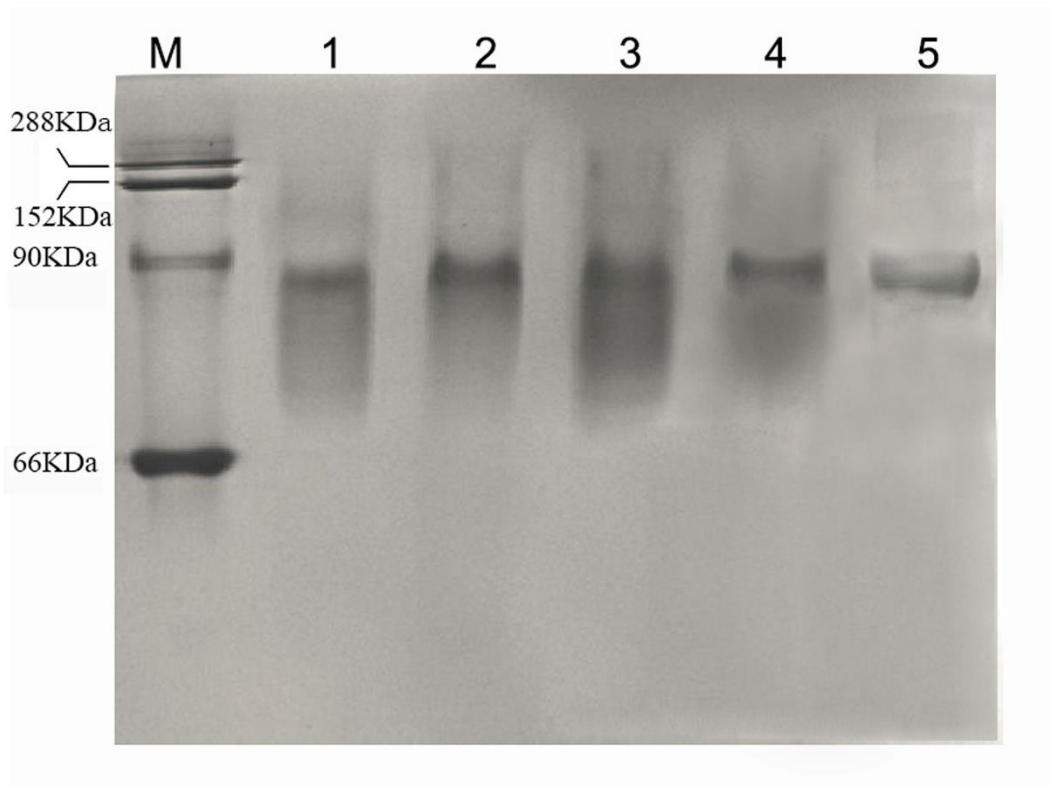
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38 **Supplementary figure 2. CD curves of *P. yanosii* CH1 L-Asnase and its single mutations.**

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42 **Supplementary figure 3. Native-PAGE analysis of *P. yayanosii* CH1 L-Asnase and its single**

43 **mutations.** Lane M: Marker. the marker was prepared by our lab. From top to bottom, the proteins, which

44 were represented by the bands, were *bacillus cereus* Leucine Dehydrogenase(288kDa), *Bacillus subtilis*

45 L-asparaginase II (152kDa), *Staphylococcus aureus* lactic dehydrogenase(90kDa), bovine serum

46 albumin(66kDa). Lane 1: *P. yayanosii* CH1 L- asparaginase. Lane 2: The mutation S17G. Lane 3: The

47 mutation A90S. Lane 4: The mutation R156S. Lane 5: The mutation K272A.

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1.seq  LPNITILATGGTIAGGGDSATKSNYTVGKVGVENLVNAVFPQIKDIANVKGEQVVNIGSQD      60
2.seq  .MRLIILGMGGTIAS...VPSEEGYES.SLSVVEILR.LAGLELKWEVEARDLLINIDSTL      54
Consensus  il  ggtia          y      ve      l      v      ni  s

1.seq  MNDNVVLTLLAKKINIDCDKTDGDFVITHGTDTEETAYFLDLTVKCD.KPVVMVGAMREPT      119
2.seq  IQPEDVWLLAETVFEAFEEFDGDFVITHGTDLAYTASMLSFVWRNPFVFIIVLTGAMREIT      114
Consensus  w  la          dg  vithgtdt  ta  l  v      p  v  gamrp  t

1.seq  SMSADGPEFNLYNAVVIADKASANRGVIVVMNITVLDGRDVIKTNITDVATEFKSVNYGPI      179
2.seq  EPGSDARRNLIWTLRFAIEGVF...GVYVAFMDKVMVIGVRVSRVRAVGLNAFQSIINYPDI      171
Consensus  d  p  nl  a  a          gv  v  d  v  g  v  k          f  s  ny

1.seq  GYIHNGKIDYQRTIARRHTSDTPFDVSKLNELEKVGIVYNYANASDLPAKALVDAGYDGI      239
2.seq  AYVKGNRVHWNAKFPKLEGEFVLDL...RHEERVLVLRVPGMEGDVLEAALELGYRGI      227
Consensusy  i      p  k      d      p  v          a      gy  gi

1.seq  VSAGVGVGNL.YKS..VEDTLATAAKTGTAVRSSRVFTGATTQDAEVD..DAKYGFVAS      294
2.seq  VLEGYGVGGIPYRGRDLLVVRRVATEIPVVMITQTLYDGVDLTKYKVGGRKALEVGVIPA      287
Consensusv  g  g  g  y      d      a      v      g      v      g

1.seq  GILNPQKARVLLQLAITQTKIPQQIQQIFNQY.....      326
2.seq  GDMTKEATITKIMWILGHTRIVGEVRRMLINMVGEIGKS      327
Consensusg          l  l  t  d

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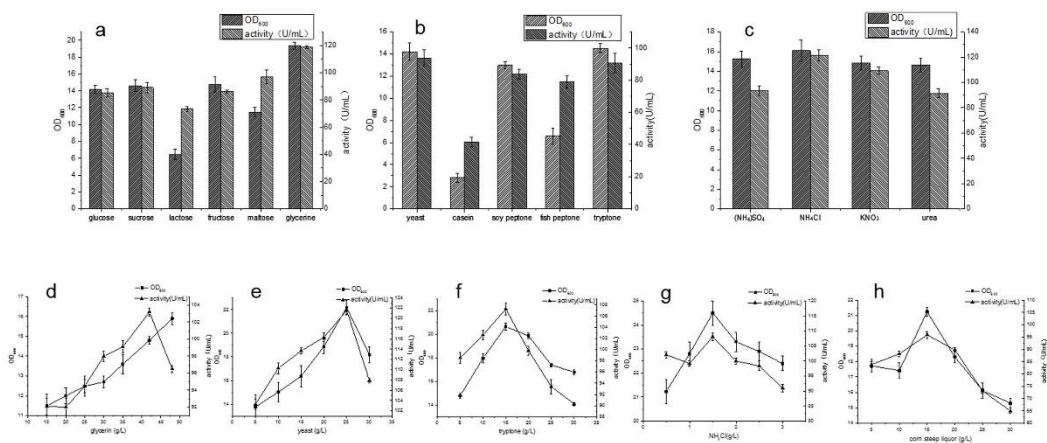
49

50 **Supplementary figure 4** Sequence alignments of *E.coil* L-asparaginase and *Pyrococcus yayanosii*

51 **CH1 L-asparaginase.** 1.seq: Sequence of *E.coil* L-asparaginase (WP\_000394140.1); 2.seq: Sequence of

52 *Pyrococcus yayanosii* CH1 L-asparaginase(WP\_013906452).

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55 **Supplementary figure 5. The effect of carbon, nitrogen, inorganic nitrogen source and corn steep**  
 56 **liquor on the biomass and L-asparaginase activity.** a) The effect of different kind carbon source with  
 57 same concentration (35g/L) on the biomass and L-asparaginase activity. b) The effect of different kind  
 58 nitrogen source with same concentration (15g/L) on the biomass and L-asparaginase. c) The effect of  
 59 different kind inorganic nitrogen source with same concentration (0.8g/L) on the biomass and L-  
 60 asparaginase. d) The effect of glycerine at different concentration (15, 20, 25, 30, 35, 42, 48g/L) on the  
 61 biomass and L-asparaginase. e) The effect of yeast at different concentration(5-30g/L) on the biomass and  
 62 L-asparaginase. f) The effect of tryptone at different concentration(5-30g/L) on the biomass and L-  
 63 asparaginase. g) The effect of NH<sub>4</sub>Cl at different concentration(0.5-3g/L) on the biomass and L-  
 64 asparaginase. h) The effect of corn steep liquor at different concentration (5-30g/L) on the biomass and L-  
 65 asparaginase.

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