

## Supplementary Data

# Hyper-production of pullulan by a novel fungus of *Aureobasidium melanogenum* ZH27 through batch fermentation

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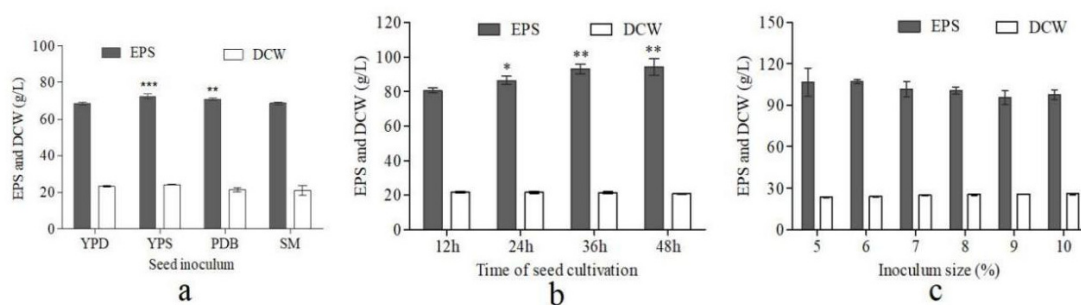
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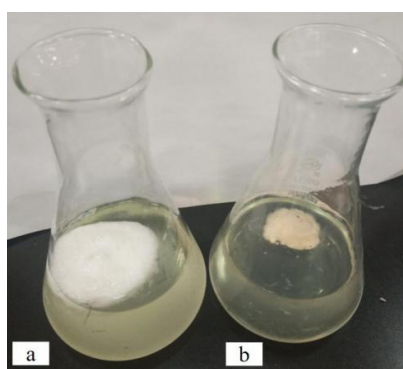
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## Supplementary Figures



**Figure S1.** Selection and optimization of seeds for producing pullulan by ZH27.  
a, seed inoculum; b: time of seed cultivation; and c: inoculum size.



**Figure S2.** Pullulan appeared in the fermented supernatants of strain ZH27 cultivating under OC (a) and IC (b) at 132 h, respectively.

## Supplementary Tables

**Table S1.** Quantitative real-time PCR primers of the genes involved in syntheses of pullulan, FOSs, trehalose, and glycerol.

| Gene name      | Primer sequence (5'→3')                             |
|----------------|---|
| <i>β-actin</i> | F: CGGTGTCACCTCACGTTGTCC<br>R: TCGAGAGCGACGTAGCAGAG |
| <i>GluT1</i>   | F: CATCTCTGCCGTCAACGCC<br>R: GAGCCAGTTGGAGGCGGTAG   |
| <i>GluT2</i>   | F: TGCGAGTGGATCGTTGGTATC<br>R: TGGAACGGATGGGAAGAGG  |
| <i>GluT3</i>   | F: CGCTGTTTCAGTTCGCTTGG<br>R: CGTATTCGTGGTTGGCGGT   |
| <i>GluT4</i>   | F: CGAAGCTGTCGCTGCATCT<br>R: GTGCCTCCGAGCAGTTGT     |

|                          |   |
|--------------------------|---|
| <i>GluT5</i>             | F: CATCGCTATGCTCATCGTCGG<br>R: GCGCCTACGAGCTTTCCTCTT    |
| <i>SUC2</i>              | F: TCTACCTCAGATACGACGCGC<br>R: GCAGGAGGTCTGGGTGTAAAG    |
| <i>GluK</i>              | F: ACTACCACGCTACCATCCGCA<br>R: CAGCATCGCAATGTCAAAGC     |
| <i>PGM</i>               | F: GGTGGTGACGGTCGTTACTGG<br>R: GATACCACCAGTGGCCTTGC     |
| <i>UGP</i>               | F: TGGAGATCATCCCCAACGG<br>R: TGACGGGAAGGAAACGACG        |
| <i>UGT1</i>              | F: TTGCAACGAACATAACCCTGGAC<br>R: AGAGCGACACCAAGACCATAAG |
| <i>CreA</i>              | F: ACCTGCCGCGTCCTTACAAGT<br>R: CTCATCGGAACGACTGAAGCG    |
| <i>Apo</i>               | F: ACTGGTGTCGGTGCCTCGTA<br>R: CCCATCTGCGAGTGTGCCT       |
| <i>GltP</i>              | F: GCAACACCCTCAGCAAGCAC<br>R: TTCTCGAGGCTGCTCAACC       |
| <i>PUL1</i>              | F: GTTGATCATGGTTGGGAAGGC<br>R: AGAGCGAAGATGGAGAAGCG     |
| $\alpha$ -amylase gene   | F: ACCTCTGGCAGCATCAGCAAT<br>R: TGGCCTTGGTAGATGATTGGG    |
| Glucosylase gene         | F: AGACTGTCAACAACCCAGCG<br>R: GTAGGCAATCATGGCAGTCGC     |
| Pullulanase gene         | F: GCAACGTCCTGGTGCAGAAAG<br>R: GACCTGGATTCGAGCCATTGC    |
| <i>Ags2</i>              | F: TCATTGCCGTCATCGTTCAGA<br>R: AGATGGGCAAGAACCAGGAGTG   |
| <i>Msn2</i>              | F: CCTCAAAAAGCAGCAGCACATC<br>R: TGATGGTAGGCTTCTGGTGTTG  |
| <i>PFK26<sup>a</sup></i> | F: GTGTTGTTATGGTCGGTCTGC<br>R: AGGATTGGGAGTGGTGGTAC     |
| <i>FBA1</i>              | F: TGGTGGTGAGGAGGATGGTGT<br>R: TTCTGGTGCTTGGAGAGGAGG    |
| <i>G3P</i>               | F: AACATCCCCTGGGCTGAGAC<br>R: GGACCTGGATGTCGGACTTGT     |
| <i>PGK</i>               | F: AGCTCGACTACTTCGCTCAGG<br>R: AAAGCCATACCACCGCAGAC     |
| <i>PYK</i>               | F: GACAAGGCCGATCTCCGTTTC<br>R: TTGACACCCTGCTGGTTCTCG    |
| <i>PYD</i>               | F: CTACACGCAGCAAAGAACCCC<br>R: CCATCACCAGCATACACCCG     |
| <i>TPS1</i>              | F: CATCAAGGGTGTTCTCAGAAGC<br>R: TTGCCGTTGATTCTGCCG      |
| <i>TPS2</i>              | F: CACATCAACCCCTGGGATCTG<br>R: GTCCATGTCTGCACAGTGTGGT   |
| <i>GPP</i>               | F: TACCAGGCAGCCGATCTATCTT<br>R: GCCCATCACCTCCAACCAT     |
| <i>GPD</i>               | F: CGCATACGACCCTCCTACCAT<br>R: TCGGTAGGCAGAGGGTAAAGC    |
| <i>Hog1</i>              | F: CCTGGTCAACGAGAAGTGC<br>R: GCTTGCCCTCGAGCATCTC        |
| <i>Sho1</i>              | F: CGCAACGTGATCGATTCTT<br>R: GTTGCTGTCCATTGCTCCCA       |
| <i>FF1</i>               | F: GGCAACTTCTCTGGCAACGAT<br>R: GTGTCTTTCTGCCTGATTCCG    |
| <i>FF2</i>               | F: TGTTCACTGGCTCAGCAGTGG<br>R: ATCACGGAAGTGAAGTCGAGCC   |
| <i>FF3</i>               | F: CAATCAAGCATTACCGTCCC<br>R: ACTCGCTTCCGACAACATGCT     |
| <i>FTR1</i>              | F: TATGCCATCCAGCGTACCG<br>R: GCAGCCAACACGAGCCTTGTT      |
| <i>AreA</i>              | F: CGGAAGTCCAAAGCCTGGTGAT<br>R: TCTTCAAACCTCAGCGGACGCAC |

*AreB*

F: CTCGTGCAAAGGAAGCATCAG  
R: ACTCAGACGGAGACGCTTAGGT

<sup>a</sup>Cited from Jiang et al. (2018)[1].

**Table S2.** Characteristic absorption peaks in the purified EPS and commercial pullulan.

| Assignments              | Wave number (cm <sup>-1</sup> ) |              |
|--------------------------|---------------------------------|--------------|
|                          | Commercial pullulan             | Purified EPS |
| –OH                      | 3,396.03                        | 3,423.03     |
| C–H                      | 2,925.48                        | 2,923.60     |
| O–C–O                    | 1,641.13                        | 1,637.29     |
|                          | 1,419.35                        | 1,427.09     |
| C–O–H                    | 1,371.14                        | 1,371.16     |
| C–O–C                    | 1,157.08                        | 1,159.03     |
| C–O                      | 1,022.09                        | 1,025.96     |
| α-1,6-glucosidic linkage | 931.45                          | 933.38       |
| α-D-glucopyranose        | 848.53                          | 852.40       |
| α-1,4-glucosidic linkage | 761.74                          | 755.97       |

**Table S3.** Comparison of pH values, pullulan titers and viscosities of supernatants at the end of fermentation of strain ZH27 under IC and OC.

| Medium           | Final pH value | Supernatant viscosity (mP·s) | Pullulan titer (g/L) |
|------------------|----------------|------------------------------|----------------------|
| Initial medium   | 2.97 ± 0.06    | 10753 ± 652.8                | 48.8 ± 3.8           |
| Optimized medium | 3.57 ± 0.06**  | 306 ± 8.54**                 | 115.4 ± 1.8**        |

## Reference

- Jiang, H.; Xue, S.-J.; Li, Y.-F.; Liu, G.-L.; Chi, Z.-M.; Hu, Z.; Chi, Z. Efficient transformation of sucrose into high pullulan concentrations by *Aureobasidium melanogenum* TN1-2 isolated from a natural honey. *Food Chem* **2018**, 257, 29-35.