Supplementary Material

**Food-grade expression of manganese peroxidases in recombinant *Kluyveromyces lactis* and degradation of aflatoxin B1 using fermentation supernatants**

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**Tables**

**Table S1.**PCR verification reaction system and programs

|  |  |  |
| --- | --- | --- |
| **PCR reaction systems** |  | **PCR reaction programs** |
| system components | content |  | temperature | time |
| ddH2O | 39.5 μL |  | Step1: 95°C | 5 min |
| 10×PCR Buffer | 5.0 μL |  | Step2: 95°C | 45 s |
| dNTPs(5.0 mmol/L) | 2.0 μL |  | Step3: 55°C | 45 s |
| upstream primer(20.0 μmol/L) | 1.0 μL |  | Step4: 72°C | 45 s |
| downstream primer(20.0 μmol/L) | 1.0 μL |  | Step5: 72°C | 10 min |
| template | 1.0 μL |  | The Step2 to Step4 was circulated for 30 rounds. |
| Taq Plus DNA Polymerase | 0.5 μL |  |

**TableS2.**Orthogonal test factors and levelsfor optimization of induction conditions

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **tests** | **A** | **B** | **C** | **D** | **E** | **F** | **G** |
| **Temperature (°C)** | **Time (h)** | **Rotation speed (rpm)** | **Hemin concentration(mmol/L)** | **pH** | **MnSO4concentration (mmol/L)** | **Galactose concentration (g/L)** |
| 1 | 28 | 84 | 180 | 0.8 | 5.5 | 0.8 | 50 |
| 2 | 30 | 96 | 200 | 1.0 | 6.0 | 1.0 | 60 |
| 3 | 32 | 108 | 220 | 1.2 | 6.5 | 1.2 | 70 |

**TableS3.** Orthogonal test factors and levels for degradation AFB1 reaction conditions

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **tests** | **A** | **B** | **C** | **D** | **E** | **F** | **G** |
| **Time (h)** | **Temperature (°C)** | **pH** | **MnSO4concentration (mmol/L)** | **Protein concentration(g/L)** | **Glucose concentration (mmol/L)** | **Glucose oxidase(U/mL)** |
| 1 | 32 | 35 | 4.2 | 0.8 | 3.0 | 2.2 | 1.0 |
| 2 | 36 | 40 | 4.5 | 1.0 | 4.0 | 2.5 | 1.2 |
| 3 | 40 | 45 | 4.8 | 1.2 | 5.0 | 2.8 | 1.5 |

**Table S4.**Orthogonal test results analysis of induced expression condition

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Experiment number** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **Degradation ratio (%)** |
| **Temperature (°C)** | **Time (h)** | **Speed(rpm)** | **Hemin (mmol/L)** | **pH** | **MnSO4 (mmol/L)** | **Galactose (g/L)** |
| 1 | 28 | 84 | 180 | 0.8 | 5.5 | 0.8 | 50.0 | 32.08 |
| 2 | 28 | 96 | 200 | 1.0 | 6.0 | 1.0 | 60.0 | 54.86 |
| 3 | 28 | 108 | 220 | 1.2 | 6.5 | 1.2 | 70.0 | 39.57 |
| 4 | 30 | 84 | 180 | 1.0 | 6.0 | 1.2 | 70.0 | 65.68 |
| 5 | 30 | 96 | 200 | 1.2 | 6.5 | 0.8 | 50.0 | 66.76 |
| 6 | 30 | 108 | 220 | 0.8 | 5.5 | 1.0 | 60.0 | 49.80 |
| 7 | 32 | 84 | 200 | 0.8 | 6.5 | 1.2 | 70.0 | 25.09 |
| 8 | 32 | 96 | 220 | 1.0 | 5.5 | 1.0 | 60.0 | 30.06 |
| 9 | 32 | 108 | 180 | 1.2 | 6.0 | 0.8 | 50.0 | 22.32 |
| 10 | 28 | 84 | 220 | 1.2 | 6.0 | 1.0 | 60.0 | 45.94 |
| 11 | 28 | 96 | 180 | 0.8 | 6.5 | 0.8 | 50.0 | 39.07 |
| 12 | 28 | 108 | 200 | 1.0 | 5.5 | 1.2 | 70.0 | 33.70 |
| 13 | 30 | 84 | 200 | 1.2 | 5.5 | 0.8 | 50.0 | 67.11 |
| 14 | 30 | 96 | 220 | 0.8 | 6.0 | 1.2 | 70.0 | 51.59 |
| 15 | 30 | 108 | 180 | 1.0 | 6.5 | 1.0 | 60.0 | 63.85 |
| 16 | 32 | 84 | 220 | 1.0 | 6.5 | 0.8 | 50.0 | 29.59 |
| 17 | 32 | 96 | 200 | 1.2 | 5.5 | 1.2 | 70.0 | 31.74 |
| 18 | 32 | 108 | 180 | 0.8 | 6.0 | 1.0 | 60.0 | 23.49 |
| k1 | 40.87 | 45.13 | 43.29 | 36.85 | 41.13 | 39.67 | 44.36 |  |
| k2 | 62.35 | 46.01 | 45.88 | 47.12 | 44.48 | 45.55 | 44.17 |  |
| k3 | 27.05 | 39.12 | 41.09 | 46.29 | 44.66 | 45.05 | 41.73 |  |
| Range R | 35.3 | 6.89 | 4.79 | 10.27 | 3.53 | 5.88 | 2.63 |  |
| Factor priority | ADBFCEG |
| optimum proposal | A2D2B2F2C2E3G1 |

**Table S5.**Orthogonal test results analysis of reaction condition

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Experiment number** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **Degradation ratio (%)** |
| **Time (h)** | **Temperature (°C)** | **pH** | **MnSO4 concentration (mmol/L)** | **Protein concentration (g/L)** | **Glucose concentration (mmol/L)** | **Glucose oxidase (U/mL)** |
| 1 | 32 | 35 | 4.2 | 0.8 | 3.0 | 2.2 | 1.0 | 37.71 |
| 2 | 32 | 40 | 4.5 | 1.0 | 4.0 | 2.5 | 1.2 | 70.03 |
| 3 | 32 | 45 | 4.8 | 1.2 | 5.0 | 2.8 | 1.5 | 27.84 |
| 4 | 36 | 35 | 4.2 | 1.0 | 4.0 | 2.8 | 1.5 | 38.64 |
| 5 | 36 | 40 | 4.5 | 1.2 | 5.0 | 2.2 | 1.0 | 73.55 |
| 6 | 36 | 45 | 4.8 | 0.8 | 3.0 | 2.5 | 1.2 | 26.42 |
| 7 | 40 | 35 | 4.5 | 0.8 | 5.0 | 2.5 | 1.5 | 64.51 |
| 8 | 40 | 40 | 4.8 | 1.0 | 3.0 | 2.8 | 1.0 | 37.62 |
| 9 | 40 | 45 | 4.2 | 1.2 | 4.0 | 2.2 | 1.2 | 56.83 |
| 10 | 32 | 35 | 4.8 | 1.2 | 4.0 | 2.5 | 1.0 | 20.94 |
| 11 | 32 | 40 | 4.2 | 0.8 | 5.0 | 2.8 | 1.2 | 48.72 |
| 12 | 32 | 45 | 4.5 | 1.0 | 3.0 | 2.2 | 1.5 | 61.92 |
| 13 | 36 | 35 | 4.5 | 1.2 | 3.0 | 2.8 | 1.2 | 68.71 |
| 14 | 36 | 40 | 4.8 | 0.8 | 4.0 | 2.2 | 1.5 | 36.20 |
| 15 | 36 | 45 | 4.2 | 1.0 | 5.0 | 2.5 | 1.0 | 60.94 |
| 16 | 40 | 35 | 4.8 | 1.0 | 5.0 | 2.2 | 1.2 | 28.33 |
| 17 | 40 | 40 | 4.5 | 1.2 | 3.0 | 2.5 | 1.5 | 75.63 |
| 18 | 40 | 45 | 4.2 | 0.8 | 4.0 | 2.8 | 1.0 | 49.99 |
| k1 | 44.53 | 43.14 | 48.72 | 43.93 | 51.35 | 49.09 | 49.09 |  |
| k2 | 50.74 | 56.97 | 69.07 | 49.58 | 45.44 | 53.10 | 53.10 |  |
| k3 | 52.17 | 47.32 | 29.56 | 53.93 | 50.65 | 45.25 | 45.25 |  |
| Range R | 7.63 | 13.83 | 39.51 | 10 | 5.91 | 7.85 | 4.01 |  |
| Factor priority | CBDFAEG |
| Optimum proposal | C2B2D3F2A3E1G3 |

**Figures**

（A）

（B）

M 1 2 3 4 5 6 7 8 M 1 2

3000

1000

500

3000

1000

500

**Figure S1.**Electrophoresis of double digestion verification of recombinant plasmids pKLAC1-Phc*mnp*, pKLAC1-Phs*mnp* and pKLAC1-Plo*mnp*plasmids extracted from the DH5α transformants. (A) Double digestion electrophoresis of recombinant pKLAC1-Phc*mnp*and pKLAC1-Phs*mnp* plasmids, lane M: DNA Marker; lanes 1~4: *Bgl*Ⅱ-*Sal*Ⅰ double digestion of pKLAC1-Phc*mnp*; lanes 5~8: *Bgl*Ⅱ-*Sal*Ⅰ double digested plasmids of pKLAC1-Phs*mnp* (B) Double digested electrophoresis of recombinant DH5α(pKLAC1-Plo*mnp*) plasmids, lane M: DNA Marker; lanes 1, 2: *Bgl*Ⅱ-*Sal*Ⅰ double digested pKLAC1-Plo*mnp.*

（A）

（B）

M 1 2 M 1 2

3000

1000

500

3000

1000

500

**Figure S2.**Electrophoresis ofPCR verificationresults fromthe recombinants GG799(pKLAC1-Phc*mnp*), GG799(pKLAC1-Phs*mnp*) and GG799(pKLAC1-Plo*mnp*). (A) lane 1: GG799 host genomic PCR verificationresults; lane 2: recombinant GG799(pKLAC1-Phc*mnp*) genomic PCR verificationresults. (B) lane 1: GG799(pKLAC1-Phs*mnp*) genomic PCR verification electrophoresis; lane 2: recombinant GG799(pKLAC1-Plo*mnp*) genomic PCR validation electrophoresis. Lane M: DNA standard molecular weight.



**B**

**A**



**D**

**C**

**Figure S3.** Degradation ratio of AFB1 by the enzyme PhcMnp under different induction conditions. (A) the effect of temperature on the degradation ratio of AFB1 by PhcMnp.(B)the effect of time on the degradation ratio of AFB1 by PhcMnp.(C)the effect of rotation speed on the degradation ratio of AFB1 by PhcMnp.(D) the effect of pH on the degradation ratio of AFB1 by PhcMnp.



**C**

**B**

**A**

**Figure S4.** Degradation ratio of AFB1 by PhcMnp under different induced additives concentrations.(A) the effect of hemin concentrations on the degradation ratio of AFB1 by PhcMnp.(B)the effect of MnSO4 concentrations on the degradation ratio of AFB1 by PhcMnp.(C)the effect of galactose concentrations on the degradation ratio of AFB1 by PhcMnp.