SUPPLEMENTARY MATERIAL TO

**Expression, purification and characterization of cellobiose dehydrogenase mutants from *Phanerochaete chrysosporium* in *Pichia pastoris* KM71H strain**

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TABLE S1.Primers used for creation of triple mutant and error prone library mutants using wtCDH – pPICZαA vector as template

|  |  |
| --- | --- |
| Primer name | Primer sequence |
| Forward primer D20N | GGTATCACCAACCCTGTTCATG |
| Forward primer A64T | CTCGGTGGCACCATGAACAAC |
| Forward primer V592M | CGCAGCCTCCATGAACTCC |
| Forward primer D20N – V22A | CACCAACCCTGCTCATGACG |
| Forward primer T84A | TTTCCTCCGCTCGCTGG |
| Forward primer A261P | ACGTATGTCCCTCCATG |
| Forward primer E674G | AGACACTCGGGGAGTACG |
| Forward primer N715S | TTGGCACGAGCAACCTGTTT |



Fig. S1. Ion-exchange chromatography of tm CDH



Fig. S2. Ion-exchange chromatography of H5 CDH



Fig. S3. Ion-exchange chromatography of H9 CDH



Fig. S4. Ion-exchange chromatography of wt CDH



Fig. S5. Gel filtration of tm CDH



Fig. S6. Gel filtration of H5 CDH



Fig. S7. Gel filtration of H9 CDH



Fig. S8. Gel filtration of wt CDH

TABLE S2 Purification table for CDH.FM-fermentation media, UF-ultrafiltrate, DEAE-sample after ion-exchange chromatography, GF-sample after gel filtration. *Cp*-protein concentration, *Ac*-enzyme activity, *V*-volume, *Y*-yield of purification, *Pf*-purification factor, *Sp*-specific enzyme activity.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | tm CDH | H5 CDH | H9 CDH | wt CDH |
|  | FM | UF | DEAE | GF | FM | UF | DEAE | GF | FM | UF | DEAE | GF | FM | UF | DEAE | GF |
| *Cp* / mg mL-1 | 0.59 | 0.42 | 0.48 | 0.08 | 0.52 | 0.45 | 0.74 | 0.17 | 0.46 | 0.20 | 0.15 | 0.024 | 0.57 | 0.72 | 0.59 | 0.22 |
| *Ac* / IU mL-1 | 0.91 | 2.34 | 5.41 | 1.17 | 1.99 | 7.30 | 16.5 | 4.89 | 0.42 | 1.10 | 1.87 | 0.34 | 4.04 | 8.86 | 8.1 | 4.49 |
| *V* / mL | 50 | 12 | 3 | 7 | 50 | 12 | 3 | 6 | 50 | 12 | 3 | 5 | 50 | 12 | 5 | 6 |
| *Y* / % | 100 | 62 | 36 | 18 | 100 | 88 | 50 | 29 | 100 | 63 | 27 | 8.2 | 100 | 53 | 20 | 14 |
| *Pf* | 1 | 3.6 | 7.2 | 9.1 | 1 | 4.2 | 5.8 | 7.3 | 1 | 5.9 | 13 | 16 | 1 | 1.7 | 1.3 | 2.88 |
| *Sp* / IU mg-1 | 1.55 | 5.55 | 11.2 | 14.1 | 3.83 | 16.2 | 22.4 | 28.1 | 0.92 | 5.44 | 12.2 | 14.5 | 7.1 | 12.3 | 13.7 | 20.4 |

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