**ADDITIONAL INFORMATION**

**Engineering *Geobacillus thermoglucosidasius* for direct utilization of holocellulose from wheat straw**

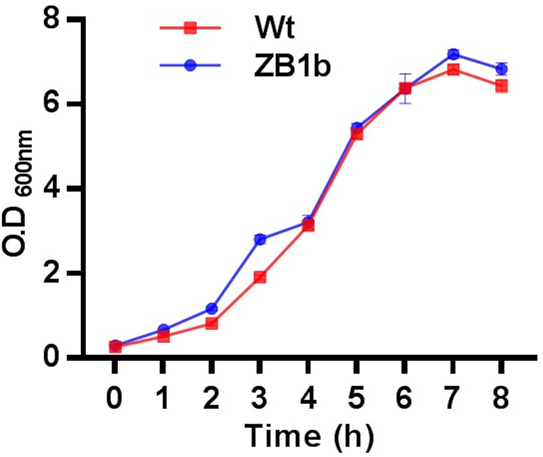
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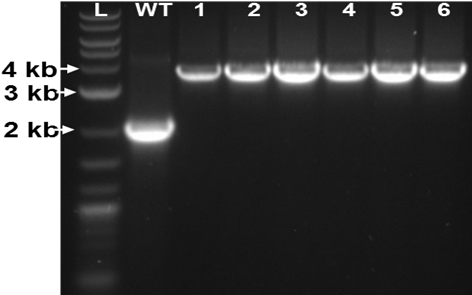
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**Figure S1**

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**Figure S1.** Growth curve analysis of wild type (WT) and recombinant *G. thermoglucosidasius* harbouringplasmid pMTLgSlimS-*CtcelA* (ZB1b). The strains were grown in 2SPYNG media at 52 ºC for 8 hours to analyse the effect of GH (CtCelA) on the growth of engineered strain.

**Figure S2**

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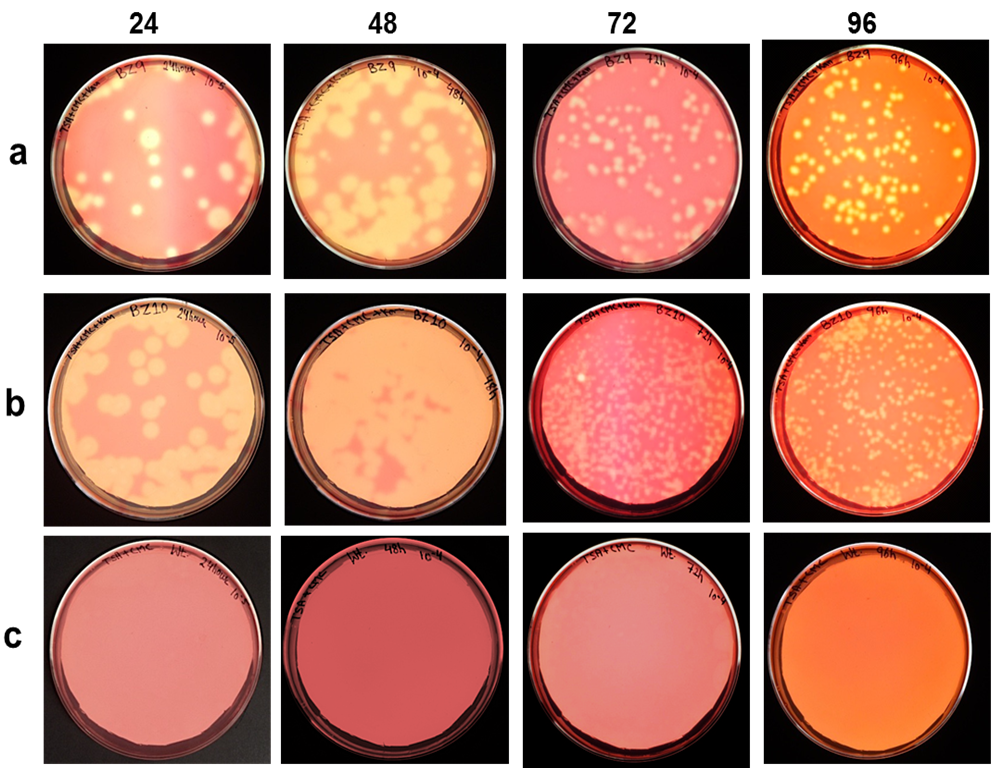
**Figure S2.** Integration of *cglT* gene at the *pyrE* locus of *G. thermoglucosidasius*.Gel depicting PCR screening of amplified product showing a band size of 2.0 kb for WT representing the *pyrE* gene and 3.8 kb (lane 1-6) for recombinant *G*. *thermoglucosidasius* ZB3bInt strains having the P*ldh*-*cglT* cassette integrated; L is DNA ladder (0.1 – 10 kb).

**Figure S3**

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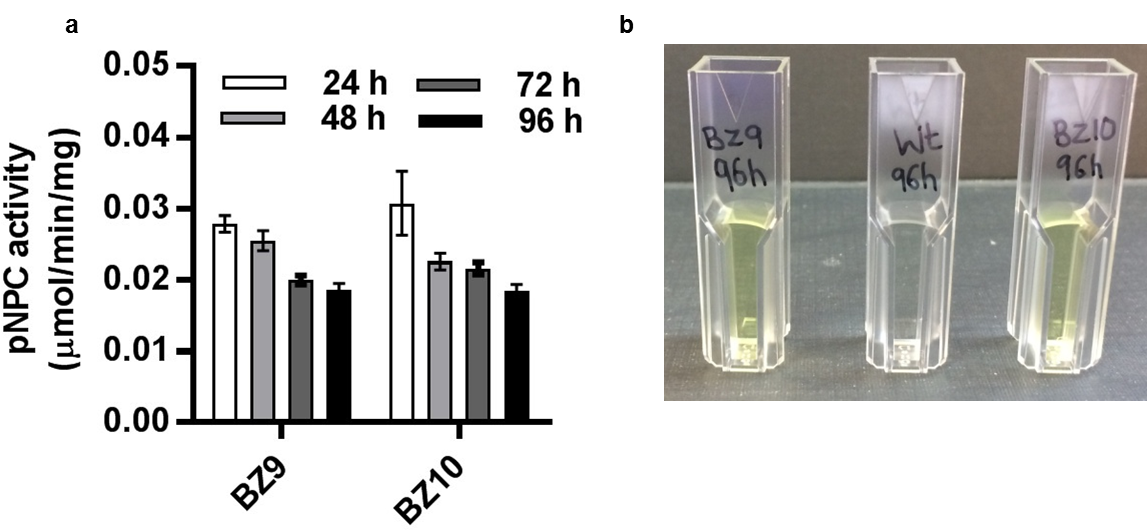
**Figure S3. The picture of pretreated wheat straw**. After a two-step process using nitric acid followed by ammonia, the pretreated wheat straw is composed of 85.15% cellulose and 4.35% xylose, of the total carbohydrates as reported in the patent (Lali, 2016). The biomass was sampled from the Cellulosic Alcohol Technology Demonstration Plant at India Glycols Ltd., Kashipur, India, Jan 2017.

**Figure S4**

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**Figure S4.** Congo red staining of recombinant *G. thermoglucosidasius* strains grown on pretreated wheat straw for 24-96 hours and plated on TSA agar supplemented with CMC for enzyme activity. (a) Recombinant *G. thermoglucosidasius* BZ9 strain expressing CglT and *Cb*CelA(b) *G. thermoglucosidasius* BZ10 strain expressing CglT, *Ct*CelA and Cel6B (c) *G. thermoglucosidasius* wild type strain.

**Figure S5**

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**Figure S5.** Colorimetric pNPC enzyme assay during growth of recombinant *G. thermoglucosidasius* BZ9 and BZ10 strains on pretreated wheat straw. (a) pNPCase specific activity of extracellular fraction of recombinant *G. thermoglucosidasius* BZ9 and BZ10 strains at 24, 48, 72, and 96 hours using pNPC (b) photograph showing pNP, a yellow colour end product liberated due to the hydrolysis of pNPC at 96 hours by *G. thermoglucosidasius* BZ9, *G. thermoglucosidasius* BZ10 and wild-type strain (Wt). Results are shown as mean ± SEM of three biological replicates.

**Table S1**

The list of primers used in this study.

|  |
| --- |
| **Name Sequence (5`-3`)** |

|  |  |
| --- | --- |
| pyrE\_C1\_F | CCCATGCTGAAAATCCAGCTG |
| pyrE\_C2\_R | CGGGTCGACAGAATTGTTCG |
| Cel6B\_F | CCCGTATTAATTTTAAAGGAGGTATAAGCTATGAGTAAAGTTCGTGCC |
| Cel6B\_R | CGACGGCCAGTGCCAAGCTTTTATTTGTCATCGTCATCTTTATAATC |
| Pldh\_CelA\_F | GTACCCGGGGATCCTCTAGAGCGGGACGGGGAGCTGAG |
| Pldh\_CelA\_R | GCTTATACCTCCTTTAAAATTAATACGGGAGGTGTGGAATGGATTTAATAAGATACCG |
| pMTLgSLimS\_HiFi\_F | TAAAAGCTTGGCACTGGCCGT |
| pMTLgSLimS\_HiFi\_R | TCTAGAGGATCCCCGGGTAC |

**File S1**

Colour coding in the nucleotide sequences denotes different parts of Bio-Brick assembled together.

Yellow; Nucleotide sequence of modified P*ldh* promoter

Red; SCAR sequence

Turquoise; Synthetic RBS

Grey; gene sequence

Pink; FLAG-epitope

P*ldh*-*CtcelA*

GCGGCCGCACTAGTGCGGGACGGGGAGCTGAGTGCTCCCGTTGTTTGCCGCGGCGTCTGTCATGAAATGGACAAACAATAGTCAAACAATCGCCACAATCGCGCATGCATTGCGGTGCGCCTTTCGCGTAAAATATTTATATGAAAGTGTTCGCAGCTAGTTTATATTGAAGGAGGATGAATGCAATGAAAAACAGGGTAATTTCATTATTAATGGCTTCCTTGCTTTTGGTTTTGTCGGTAATTGTTGCTCCTTTTTACAAAGCGGAAGCCGCGGGAGTGCCGTTTAATACGAAATATCCGTATGGACCTACGTCCATTGCGGATAATCAATCGGAAGTAACAGCGATGTTGAAAGCGGAATGGGAAGATTGGAAAAGCAAACGCATTACGAGCAATGGCGCGGGAGGATACAAACGGGTTCAACGTGATGCTAGTACAAATTATGATACAGTGTCCGAAGGCATGGGCTATGGCTTGCTTCTTGCGGTCTGCTTTAACGAACAAGCGTTGTTTGATGATCTTTATCGCTATGTTAAAAGCCATTTTAATGGCAATGGCCTTATGCATTGGCATATTGATGCGAATAACAATGTCACATCCCATGATGGGGGGGATGGAGCGGCGACAGATGCGGATGAAGATATTGCGTTAGCGCTTATTTTTGCGGATAAACTTTGGGGCAGCTCCGGCGCGATTAATTATGGACAAGAAGCGCGCACACTTATTAACAACTTGTATAATCATTGTGTAGAACATGGCTCGTATGTCTTGAAACCGGGCGATCGCTGGGGAGGCAGCTCGGTCACGAATCCTTCGTATTTTGCGCCAGCGTGGTATAAGGTCTATGCGCAATATACGGGGGATACACGCTGGAATCAAGTCGCGGATAAATGCTATCAAATTGTAGAAGAAGTTAAAAAATATAACAACGGCACAGGCTTAGTACCTGATTGGTGCACAGCGTCCGGCACGCCTGCGTCCGGCCAAAGCTATGATTACAAGTATGATGCGACACGGTATGGCTGGCGTACAGCGGTAGATTATAGCTGGTTTGGAGATCAACGTGCGAAAGCGAATTGTGATATGTTGACAAAATTCTTTGCGAGAGATGGAGCGAAAGGCATTGTCGATGGCTATACGATTCAAGGCTCCAAAATTTCCAACAACCATAATGCGTCGTTTATTGGACCAGTCGCGGCGGCGAGCATGACGGGCTATGATTTGAATTTTGCGAAAGAACTTTATAGAGAAACAGTCGCGGTCAAAGATTCGGAATATTATGGCTATTATGGAAATTCCCTTCGGTTACTTACGTTGTTGTATATTACGGGCAATTTTCCTAATCCACTTTCCGATCTTTCGGGCCAACCGACGCCACCTAGCAATCCTACACCTAGCCTTCCACCTCAAGTAGTCTATGGAGATGTCAATGGCGATGGCAATGTGAATAGCACGGATCTTACAATGTTGAAACGCTATCTTCTTAAATCGGTGACGAATATTAATCGTGAAGCGGCGGATGTGAATAGAGATGGCGCGATTAATTCCTCCGATATGACGATTCTTAAACGGTATCTTATTAAATCCATTCCACACCTCCCGTATGATTATAAAGATGACGATGACAAATAA

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