Supplementary belongs to:

Biochemical characterization of the functional roles of residues in the active site of the β-galactosidase from *Bacillus circulans* ATCC 31382

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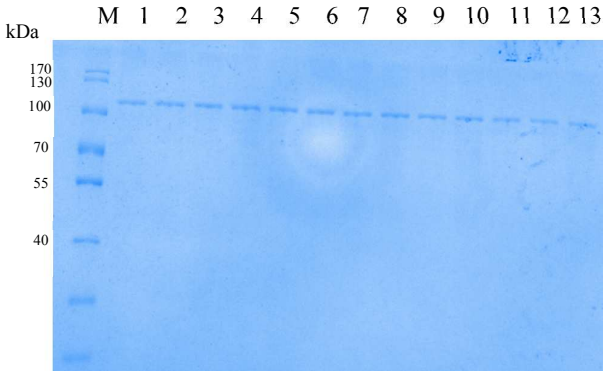
Table S1. The primer pairs used for site-directed mutagenesis of BgaD-D.

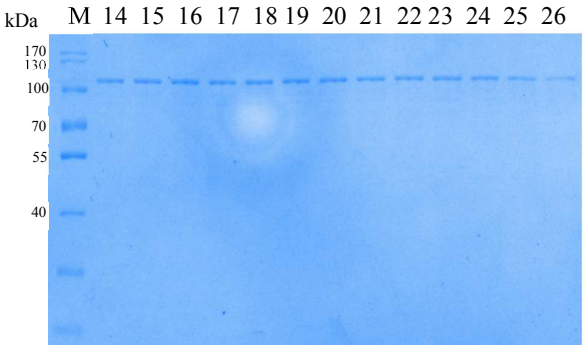
Primer name	DNA sequence (5' to 3')
Arg185-F	CACCCAGCCGAGCAGCNNKTGGTATTCGGGGAGCG
Arg185-R	CGCTCCCGAATACCAMNNGCTGCTCGGCTGGGTG
Asp481-F	GATCGGCGAGNNKAAAACCC
Asp481-R	GGGTTTTMNNCTCGCCGATC
Asp481Lys-F	GATCGGCGAGAAGAAAACCC
Asp481Lys-R	GGGTTTTCTTCTCGCCGATC
Asp481Arg-F	GATCGGCGAGCGGAAAACCC
Asp481Arg-R	GGGTTTTCCGCTCGCCGATC
Asp481Asn-F	GATCGGCGAGAACAAAACCC
Asp481Asn-R	GGGTTTTGTTCTCGCCGATC
Asp481Gln-F	GATCGGCGAGCAGAAAACCC
Asp481Gln-R	GGGTTTTCTGCTCGCCGATC
Asp481Leu-F	GATCGGCGAGCTGAAAACCC
Asp481Leu-R	GGGTTTTCAGCTCGCCGATC
Asp481Trp-F	GATCGGCGAGTGGAAAACCC
Asp481Trp-R	GGGTTTTCCACTCGCCGATC
Asp481Gly-F	GATCGGCGAGGGGAAAACCC
Asp481Gly-R	GGGTTTTCCCCTCGCCGATC
Lys487-F	CGCGGAGACNNKGTAAATGTTACAC
Lys487-R	GTGTAACATTTACMNNGTCTCCGCG
Tyr511-F	GGACTGAACNNKAGCGAGAACAACTATGATGGC
Tyr511-R	GCCATCATAGTTGTTCTCGCTMNNGTTCAGTCC
Tyr511Phe-F	GGACTGAACTATAGCGAGAACAACTATGATGGC
Tyr511Phe-R	GCCATCATAGTTGTTCTCGCTATAGTTCAGTCC
Tyr511Trp-F	GGACTGAACTGGAGCGAGAACAACTATGATGGC
Tyr511Trp-R	GCCATCATAGTTGTTCTCGCTCCAGTTCAGTCC
Trp570-F	GTCGGCNNKGGACGAACTGCAGAAG
Trp570-R	CTTCTGCAGTTCGTCCMNNGCCGAC
Trp570Tyr-F	GTCGGCTACGGACGAACTGC
Trp570Tyr-R	GCAGTTCGTCCGTAGCCGAC
Trp570Phe-F	GTCGGCTTTGGACGAACTGC
Trp570Phe-R	GCAGTTCGTCCAAAGCCGAC
Trp570Ala-F	GTCGGCGCTGGACGAACTGC
Trp570Ala-R	GCAGTTCGTCCAGCGCCGAC
Trp570Val-F	GTCGGCGTTGGACGAACTGC
Trp570Val-R	GCAGTTCGTCCAACGCCGAC
Trp570Leu-F	GTCGGCCTTGGACGAACTGC
Trp570Leu-R	GCAGTTCGTCCAAGGCCGAC
Trp570Cys-F	GTCGGCTGCGGACGAACTGC
Trp570Cys-R	GCAGTTCGTCCGCAGCCGAC
Trp593-F	ACCTGAAGCATATTGCAGGGCAATTTATCNNKACCGGCTTTGATTATATTGG
Trp593-R	CCAATATAATCAAAGCCGGTMNNGATAAATTGCCCTGCAATATGCTTCAGGT
Glu601-F	CCGGCTTTGATTATATTGGCNNKCCGACGCCATATTATAATTCC

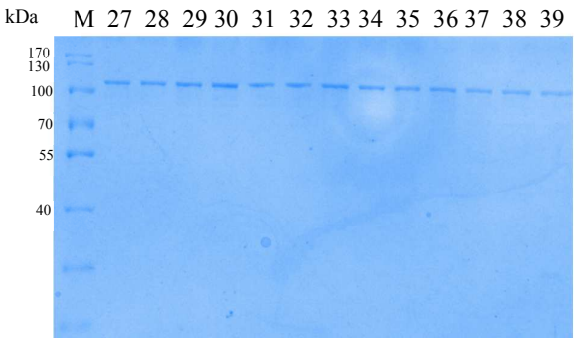
Glu601-R	GGAATTATAATATGGCGTCGGMNNGCCAATATAATCAAAG CCGG
Glu601Asp-F	CCGGCTTTGATTATATTGGCGACCCGACGCCATATTATAAT TCC
Glu601Asp-R	GGAATTATAATATGGCGTCGGGTCGCCAATATAATCAAAG CCGG
Phe616-F	GCAAAAAGCTCCTATNNKGGTGCTGTGGATACGG
Phe616-R	CCGTATCCACAGCACCMNNATAGGAGCTTTTTGC
Phe616Trp-F	GCAAAAAGCTCCTATTGGGGTGCTGTGGATACGG
Phe616Trp-R	CCGTATCCACAGCACCCCAATAGGAGCTTTTTGC
Phe616His-F	GCTCCTATCATGGTGCTGTGGATAC
Phe616His-R	GTATCCACAGCACCATGATAGGAGC
Phe616Ser-F	GCTCCTATTCAGGTGCTGTGGATAC
Phe616Ser-R	GTATCCACAGCACCTGAATAGGAGC
Phe616Thr-F	GCTCCTATACAGGTGCTGTGGATAC
Phe616Thr –R	GTATCCACAGCACCTGTATAGGAGC
Phe616Asn-F	GCTCCTATAATGGTGCTGTGGATAC
Phe616Asn-R	GTATCCACAGCACCATTATAGGAGC
Phe616Cys-F	GCTCCTATTGCGGTGCTGTGGATAC
Phe616Cys-R	GTATCCACAGCACCGCAATAGGAGC
Phe616Pro-F	GCTCCTATCCAGGTGCTGTGGATAC
Phe616Pro-R	GTATCCACAGCACCTGGATAGGAGC
Phe616Ala-F	GCTCCTATGCTGGTGCTGTGGATAC
Phe616Ala-R	GTATCCACAGCACCAGCATAGGAGC
Phe616Ile-F	GCTCCTATATCGGTGCTGTGGATAC
Phe616Ile-R	GTATCCACAGCACCGATATAGGAGC
Phe616Met-F	GCTCCTATATGGGTGCTGTGGATAC
Phe616Met-R	GTATCCACAGCACCCATATAGGAGC
Phe616Tyr-F	GCTCCTATTACGGTGCTGTGGATAC
Phe616Tyr-R	GTATCCACAGCACCGTAATAGGAGC

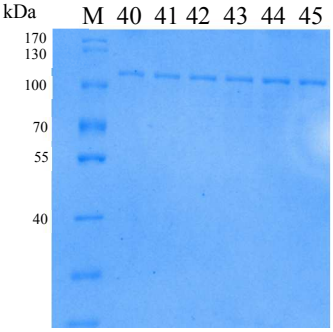
The “N” base in the primers stands for a “A or T or C or G” base, and “K” stands for a “G or T” base. “NNK” codons are commonly used in screens for codon substitutions to reduce the presence of some codon-rich amino acids, thereby reducing their over representation in a particular library. Additionally, using NNK codons removes 2 out of 3 possible stop codons, which also limits the number of sequences in a library that produce unwanted truncated gene products[1].

Figure S1. SDS-PAGE gel analysis of the wild-type BgaD-D and the mutant enzymes (loaded with 5 μL of 0.1 mg/mL protein solutions). M, Marker proteins; 1, WT; 2, Trp570Gly; 3, Trp570Thr; 4, Trp570Arg; 5, Trp570Glu; 6, Trp570Tyr; 7, Trp570Phe; 8, Trp570Ala; 9, Trp570Val; 10, Trp570Cys; 11, Trp570Leu; 12, Trp593Tyr; 13, Trp593Phe; 14, Phe616Val; 15, Phe616Glu; 16, Phe616Gly; 17, Phe616Lys; 18, Phe616Gln; 19, Phe616Arg; 20, Phe616Asp; 21, Phe616Leu; 22, Phe616Trp; 23, Phe616His; 24, Phe616Ser; 25, Phe616Thr; 26, Phe616Asn; 27, Phe616Cys; 28, Phe616Pro; 29, Phe616Ala; 30, Phe616Ile; 31, Phe616Met; 32, Phe616Tyr; 33, Asp481Glu; 34, Asp481His; 35, Asp481Ser; 36, Asp481Asn; 37, Asp481Gln; 38, Lys487Met; 39, Lys487Phe; 40, Lys487Leu; 41, Lys487Gln; 42, Lys487Ser; 43, Lys487Gly; 44, Lys487Asn; 45, Lys487Cys.









Reference:

1 Tang, L., Gao, H., Zhu, X., Wang, X., Zhou, M., and Jiang, R. (2012) Construction of "small-intelligent" focused mutagenesis libraries using well-designed combinatorial degenerate primers. *Biotechniques*, *52* (3), 149–158.