

Table S1 Primers used in this study

| Published Name | Plasmid | Primer Sequences (5'-3') |
|--|-----------------|--|
| For construction of the GenR expression plasmid | | |
| ncgl2921e1 | pZWHJ003 | CGCGGATCCGATAACGTCGCCCCCACC |
| ncgl2921e2 | | CCCAAGCTTTTCACCCTTTTTTCGGGCACTC |
| ncgl2921e3 | pZWHJ001 | CCCAAGCTTAAAGGAGGACAACCGTGATTATGGATAACGTCGCC |
| ncgl2921e4 | | CGCGGATCCTCACCCCTTTTTTCGGGCACTC |
| laczf | pZWHJ002 | CCCAAGCTTCAGCTATGACCATGATTACGG |
| laczr | | TTGGGCGCCCTTACGCGAAATACGGGCAG |
| ptacr3 | pZWHJ005 | TGCTCTAGATATCGACTGCACGGTGCAC |
| ptacr4 | | TGCTCTAGATTCTGTTTCCTGTGTGAAATTG |
| For RT-PCR analysis | | |
| prgenMF01 | MFp | CATGAGGTTGCACAGTGCGATG |
| prgenMF02 | | TGGCGACGTCATTGTCACCG |
| prgenDF01 | DFp | GCTGGA ACTACTGCATCTAGC |
| prgenDF02 | | GAATATCGTCGCTGGCTCTGAC |
| prgenRD01 | RDp | CAATCCCACTTGTGTGCCAC |
| prgenRD02 | | TATGCGGGATCTGAACAAGGC |
| prgenKH01 | KHp | TCATTGCAGCTGTCGCCAAC |
| prgenKH02 | | AGCGCAAGTGCACCTGCTGC |
| For RT-qPCR analysis | | |
| ptgenIKL01 | DFM | ATGGGATATCGAGCCCGTCTAG |
| pgenDFM01 | | TGTTATCGAAGGCGAAGGCG |
| ptgenR06 | R-Wild | TGGAAGGTAGGTGCGGGAC |
| ptgenR07 | | TTGCTGCTCCGTGACTCTGG |
| ptgenR03 | R-mutant | TCGGGCACTCGGTAATCAGC |
| ptgenR10 | | CCCGAAAGAATTTCTAAGCTC |
| ptgenTX03 | KH | GCCGAACTCATAACCTGCAG |
| pgenKH01 | | TGAGCCAGCAGGTTTCAAGG |
| pcg16S05 | 16S rDNA | GAGGAACACCAATGGCGAAG |
| pcg16S06 | | CGGCACAGAAGTCGTGGAAG |
| For construction of the promoter-cloning plasmids | | |
| pgenDFM01 | pZWHJ006 (DFMa) | CGCGGATCCACTCAACGATGTGGGTGATG |
| pgenDFM02 | | CCCAAGCTTCAGCTCTGCCTGCTCTTCTG |
| pgenR01 | pZWHJ007 (Ra) | CGCGGATCCTCGCCATGCCAAAGAACG |
| pgenR02 | | CCCAAGCTTGCACCGGAAATGGTCAAAC |
| pgenKH01 | pZWHJ008 (KHa) | CCCAAGCTT GCCAATGAGCACCGCAGCCAAG |
| pgenKH02 | | CGCGGATCCTGGAAGGTAGGTGCGGGAC |

For S1 Nuclease protection mapping and DNase I footprinting of *genDFM*, *genR* and *genKH*

| | | |
|----------------------|------|------------------------------|
| pegenDFM01 (DFM up) | DFMb | TGCTTGACGTGTTTCAGTTGC |
| prgenRD02 (DFM down) | | TATGCGGGATCTGAACAAGGC |
| pegenR01 (R up) | Rb | TGAGCTGCAATGCAATGTTCG |
| pgenR01 (R down) | | CGCGGATCCTCGCCATGCCAAAGAACG |
| pegenKH04 (KH up) | KHb | GCCAATGAGCACCGCAGCCAAG |
| pgenKH02 (KH down) | | CGCGGATCCTGGAAGGTAGGTGCGGGAC |

For Primer extension and DNA sequencing ladder of *genDFM*, *genR* and *genKH*

| | |
|------------|------------------------|
| pegenDFM01 | TGCTTGACGTGTTTCAGTTGC |
| pegenR01 | TGAGCTGCAATGCAATGTTCG |
| pegenKH04 | GCCAATGAGCACCGCAGCCAAG |

For EMSA

| | | |
|------------|---------------------|----------------------------------|
| pegenDFM04 | DFMan | CCCAAGCTTGCGCCATTGTTGGAGTCC |
| pegenDFM09 | | CGCGGATCCATGGGGGGAATTTTCAGAGCTG |
| pegenR04 | R-KHan | CCCAAGCTTGCGGACGTTATCCATAATC |
| pegenKH07 | | CCCAAGCTTGCCAATGAGCACCGCAGCCAAG |
| pegenDFM10 | DFMan01 | CGCGGATCCGATTCCACATAGCGGAATTATC |
| pegenDFM12 | DFMan01m | CCCAAGCTTCTGGAGTTGCGGGTGGGACTTC |
| pegenDFM13 | | CGCGGATCCGCGTTACATAGCTTGCCTATC |
| pegenDFM12 | DFMan02 | CCCAAGCTTCTGGAGTTGCGGGTGGGACTTC |
| pegenDFM11 | | CCCAAGCTTTAAGTATTCCCCTCAGGAAAC |
| pegenDFM01 | DFMan02m | CGCGGATCCACTCAACGATGTGGGTGATG |
| pegenDFM14 | | CCCAAGCTTTAAGT GGCAACCTCAAACGGC |
| pegenDFM01 | R-KHan | CGCGGATCCACTCAACGATGTGGGTGATG |
| pegenR04 | | CCCAAGCTTGCGGACGTTATCCATAATC |
| pegenKH07 | R-KHan01, R-KHan01m | CCCAAGCTTGCCAATGAGCACCGCAGCCAAG |
| pegenR08 | | CCCAAGCTTGAGCCAAGTTACAATCAAAG |
| pegenR09 | R-KHan02, R-KHan02m | CGCGGATCCAAACCCCGGTAGACCAGCATTG |
| pegenKH08 | | CGCGGATCCCACCTCGATTATGTGTTTATGAC |
| pegenKH10 | | CCCAAGCTTGGGCCGATTGGACGGTGCC |

For mutated GenR binding sites

| | | |
|-------------|--------------|---|
| pDFMnmut01 | pZWHJ009 | GTTACATAGCTTGCCTATCTTGAGTCACCTTTCAAAGC |
| pDFMnmut02 | | GGCAAGCTATGTAACGGCTAAGTATCCCCTCAGGAAACG |
| pDFMnmut03 | pZWHJ010 | CCGTTTGAGGTTGCCACTTAGATTCCACATAGCGG |
| pDFMnmut04 | | GGCAACCTCAAACGGCGGGGGTGTGACAGCTCTG |
| pDFMnmut04 | pZWHJ011 | GGCAACCTCAAACGGCGGGGGTGTGACAGCTCTG |
| pDFMnmut05 | | CCGTTTGAGGTTGCCACTTAGCCGTTACATAGCTTGC |
| pR-KHnmut01 | pZWHJ012 and | GTTGCTATCTTTGCCGAGTGATTATGGATAACGTC |
| pR-KHnmut02 | pZWHJ013 | GGCAAAGATAGCAACGGAATCCCTTGTATTGTAACCTGG |
| pR-KHnmut03 | pZWHJ014 and | GTTGGTGC GTTTGCCGTCATAAACACATAATCGAGG |
| pR-KHnmut04 | pZWHJ015 | GGCAAACGCACCAACGGCGGGAGGACTTGTGACAG |

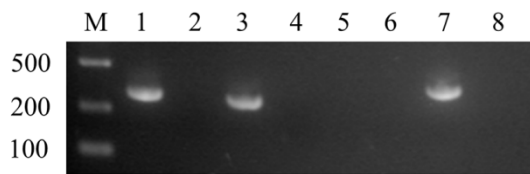


FIG S1 Transcriptional analysis of the *gen* cluster of *C. glutamicum* by RT-PCR. Lanes 1, 3, 5, and 7 show the amplified DNA fragments, here called MFp, DFp, RDp, and KHp in Fig. 1B. Lanes 2, 4, 6, and 8 show their corresponding negative controls without reverse transcriptase. No products were detected in controls and RDp amplification.

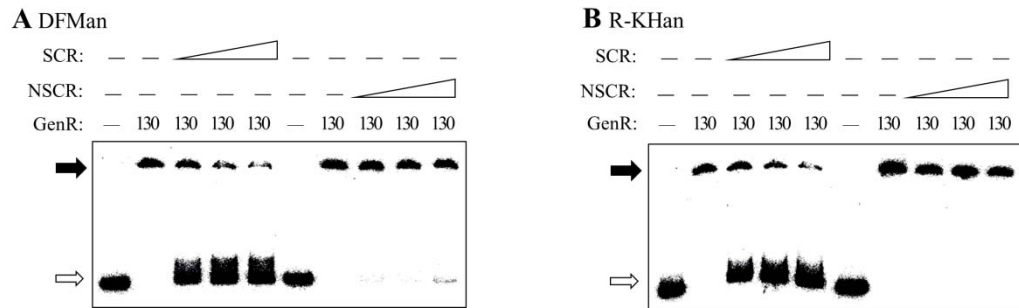


FIG S2 Electrophoretic mobility shift assays for determination of GenR binding sites upstream of *genDFM*, *genR*, and *genKH* operons. A and B. Competition assays using unlabeled specific competitor (SCR) and non-specific competitor (NSCR) DNA. Labeled probe and unlabeled competitor of 25-fold, 50-fold, and 100-fold more in amount were incubated with 130nM His₆-GenR and 300 nM 3-HBA for 30 min at 25°C. Each lane contained 0.5 ng (0.3-0.4nM) of ³²P-labeled DFMan and R-KHan probes. The free probes are indicated by open arrows and the retarded DNA fragments by solid arrows.

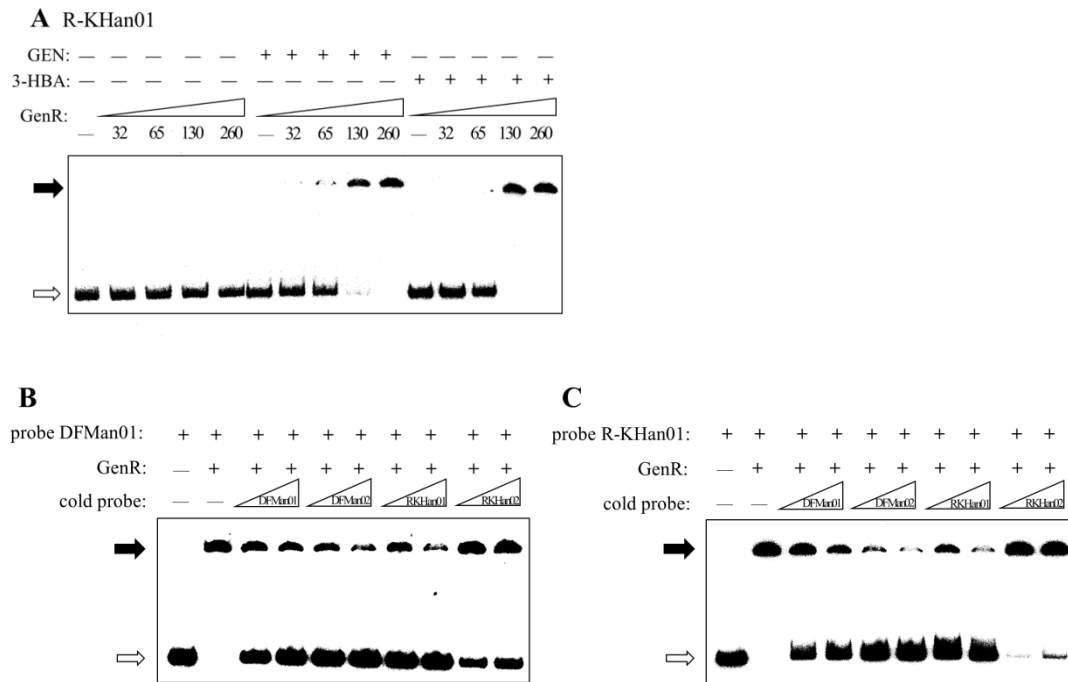


FIG S3 EMSAs for determination of GenR binding sites. A: Effects of 3-HBA and GEN on the binding affinity of GenR for the site R-KH01. The concentrations (nM) of purified His₆-GenR are indicated by numbers under parentheses, with 300 nM GEN and 3-HBA (+) or without (-). B and C: Comparison of the relative affinity of GenR to the binding sites DFMan01 and R-KHan01. Labeled probe and the unlabeled competitors were incubated with 130 nM His₆-GenR and 300 nM 3-HBA for 30 min at 25°C. The free probes are indicated by open arrows and the retarded DNA fragments are indicated by solid arrows.