



Fig. S1. Evolutionary distance tree based on Penicillin Binding Proteins (PBPs) from different microorganisms. The tree was constructed by neighbour joining method using 49 PBPs. Numbers represent percentages of 1000 bootstraps.

Table S1. Bacterial strains and plasmids

| <u>Strains or Plasmids</u> | <u>Relevant genotype or description</u> | <u>Source or Ref</u> |
|----------------------------------|---|--------------------------------|
| <u>Strains</u> | | |
| <i>E. coli</i> DH5 α | r- m- strain used for general cloning | (Hanahan, 1983) |
| <i>E. coli</i> S17-1 | Mobilizing donor strain, <i>pro recA</i> , which has the RP4 derivative <i>tra</i> system integrated into the chromosome | (Schafer <i>et al.</i> , 1990) |
| <i>E. coli</i> XL1-Blue MRF' | Host strain used for propagating the pBT and pTRG recombinant plasmids for the Two hybrid system. | Stratagene |
| <i>E. coli</i> BacterioMatch® II | Reporter strain derived from Stratagene's XL1-Blue MR, harbours <i>lacI^f</i> and the reporter genes <i>His3</i> and <i>aadA</i> . Used for Two hybrid system analysis | Stratagene |
| <i>C. glutamicum</i> 13032 | Wild-type | ATCC |
| <i>C. glutamicum</i> RES167 | 13032 derivative used as host for transformation, electroporation or conjugation | (Tauch <i>et al.</i> , 2002) |
| <i>C. glutamicum</i> RES1A | <i>pbp1a</i> mutant caused by integration of plasmid pK1Aint into the chromosome of <i>C. glutamicum</i> RES167. Kan ^R | This work |
| <i>C. glutamicum</i> RES1B | <i>pbp1b</i> mutant caused by integration of plasmid pK1Bint into the chromosome of <i>C. glutamicum</i> RES167. Kan ^R | This work |
| <i>C. glutamicum</i> RES2A | <i>pbp2a</i> mutant caused by integration of plasmid pK2Aint into the chromosome of <i>C. glutamicum</i> RES167. Kan ^R | This work |
| <i>C. glutamicum</i> RES2B | <i>pbp2b</i> mutant caused by integration of plasmid pK2Bint into the chromosome of <i>C. glutamicum</i> RES167. Kan ^R | This work |
| <i>C. glutamicum</i> RES1A1B | <i>pbp1a+pbp1b</i> mutant caused by integration of plasmid pOJP1Bint into the chromosome of <i>C. glutamicum</i> RES1A. Kan ^R Apr ^R | This work |
| <i>C. glutamicum</i> RES1A2A | <i>pbp1a+pbp2a</i> mutant caused by integration of plasmid pOJP2Aint into the chromosome of <i>C. glutamicum</i> RES1A. Kan ^R Apr ^R | This work |
| <i>C. glutamicum</i> RES1A2B | <i>pbp1a+pbp2b</i> mutant caused by integration of plasmid pOJP2Bint into the chromosome of <i>C. glutamicum</i> RES1A. Kan ^R Apr ^R | This work |
| <i>C. glutamicum</i> RES1B2A | <i>pbp1b+pbp2a</i> mutant caused by integration of plasmid pOJP2Aint into the chromosome of <i>C. glutamicum</i> RES1B. Kan ^R Apr ^R | This work |
| <i>C. glutamicum</i> RES2A2B | <i>pbp2a+pbp2b</i> mutant caused by integration of plasmid pOJP2Bint into the chromosome of <i>C. glutamicum</i> RES2A. Kan ^R Apr ^R | This work |
| <i>C. glutamicum</i> RESK2B | Obtained by integration of plasmid pOK2B into RES167; contains a full copy of <i>pbp2b</i> under <i>PgntK</i> (<i>Pkan-pbp2b</i>). Apr ^R | This work |
| <i>C. glutamicum</i> RES1BK2B | Obtained by integration of plasmid pOK2B into strain RES1B; contains a chromosomal disrupted copy of <i>pbp1b</i> on its original position and a copy of <i>pbp2b</i> under <i>PgntK</i> (<i>Pkan-pbp2b</i>). Kan ^R Apr ^R | This work |
| <i>C. glutamicum</i> KG1A | Obtained by integration of plasmid pOJKG1A into RES167; contains a chromosomal disrupted copy of <i>pbp1a</i> on its original position and a copy of <i>gfp-pbp1a</i> under <i>Pkan</i> . Apr ^R | This work |
| <i>C. glutamicum</i> KG1B | Obtained by integration of plasmid pOJKG1B into RES167; contains a chromosomal disrupted copy of <i>pbp1b</i> on its original position and a | This work |

| | | |
|---------------------------|--|---------------------------------|
| | copy of <i>gfp-pbp1b</i> under <i>Pkan. Apr^R</i> | |
| <i>C. glutamicum</i> KG2A | Obtained by integration of plasmid pOJKG2A into RES167; contains a chromosomal disrupted copy of <i>pbp2a</i> on its original position and a copy of <i>gfp-pbp2a</i> under <i>Pkan. Apr^R</i> | This work |
| <i>C. glutamicum</i> KG2B | Obtained by integration of plasmid pOJKG2B into RES167; contains a chromosomal disrupted copy of <i>pbp2b</i> on its original position and a copy of <i>gfp-pbp2a</i> under <i>Pkan. Apr^R</i> | This work |
| Plasmids | | |
| pGEM-TEasy | <i>E. coli</i> vector containing <i>lacI</i> , <i>ori1</i> and <i>bla</i> gene | Promega |
| pG1A | pGEM-TEasy derivative containing a 2.4-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>pbp1a</i> gene. | This work |
| pG1B | pGEM-TEasy derivative containing a 2.2-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>pbp1b</i> gene. <i>bla</i> | This work |
| pG2A | pGEM-TEasy derivative containing a 1.9-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>pbp2a</i> gene. <i>bla</i> | This work |
| pG2B | pGEM-TEasy derivative containing a 1.5-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>pbp2b</i> gene. <i>bla</i> | This work |
| pXHis1-Npro | <i>E. coli</i> vector containing the <i>xysA</i> gene from <i>Streptomyces halstedii</i> under <i>Pkan. bla</i> | (Adham <i>et al.</i> , 2001) |
| pK1A, 1B, 2A, 2B serie | pXHis1-Npro derivative plasmids obtained by replacement of the <i>xysA</i> gene (<i>NdeI-XhoI</i>) by the full PCR-amplified <i>pbp</i> gene | This work |
| pABK | Mobilizable bifunctional plasmid <i>E. coli</i> and pBL1 origin of replication) and the <i>kan</i> resistance determinant | (Valbuena <i>et al.</i> , 2006) |
| pAK1A | pABK derivative containing the <i>BglII</i> cassette including <i>pbp1a</i> under <i>Pkan</i> and flanked by transcriptional terminators T1 and T2. <i>kan</i> | This work |
| pAK1B | pABK derivative containing the <i>BglII</i> cassette including <i>pbp1b</i> under <i>Pkan</i> and flanked by transcriptional terminators T1 and T2. <i>kan</i> | This work |
| pAK2A | pABK derivative containing the <i>BglII</i> cassette including <i>pbp2a</i> under <i>Pkan</i> and flanked by transcriptional terminators T1 and T2. <i>kan</i> | This work |
| pAK2B | pABK derivative containing the <i>BglII</i> cassette including <i>pbp2b</i> under <i>Pkan</i> and flanked by transcriptional terminators T1 and T2. <i>kan</i> | This work |
| pK18mob | <i>E. coli</i> mobilizable plasmid containing the <i>kan</i> resistance determinant | (Schäfer <i>et al.</i> , 1994) |
| pK1Aint | pK18mob derivative containing an internal 550-bp fragment of <i>C. glutamicum pbp1a</i> obtained by PCR amplification. <i>kan</i> | This work |
| pK1Bint | pK18mob derivative containing an internal 460-bp fragment of <i>C. glutamicum pbp1b</i> obtained by PCR amplification. <i>kan</i> | This work |
| pK2Aint | pK18mob derivative containing an internal 680-bp fragment of <i>C. glutamicum pbp2a</i> obtained by PCR amplification. <i>kan</i> | This work |
| pK2Bint | pK18mob derivative containing an internal 670-bp fragment of <i>C. glutamicum pbp2b</i> obtained from pG2B. <i>kan</i> | This work |

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| pOJ260 | <i>E. coli</i> mobilizable plasmid containing the <i>apr</i> resistance marker | (Bierman <i>et al.</i> , 1992) |
| pOJ1Aint | pOJ260 derivative containing an internal 550-bp fragment of <i>C. glutamicum pbp1a</i> obtained by PCR amplification. <i>apr</i> | This work |
| pOJ1Bint | pOJ260 derivative containing an internal 460-bp fragment of <i>C. glutamicum pbp1b</i> obtained by PCR amplification. <i>apr</i> | This work |
| pOJ2Aint | pOJ260 derivative containing an internal 680-bp fragment of <i>C. glutamicum pbp2a</i> obtained by PCR amplification. <i>apr</i> | This work |
| pOJ2Bint | pOJ260 derivative containing an internal 670-bp fragment of <i>C. glutamicum pbp2b</i> obtained from pG2B. <i>apr</i> | This work |
| pOKD | pOJ260 derivative containing the 5'-end of <i>C. glutamicum divIVA</i> under the control of the promoter of the <i>C. glutamicum gntK</i> gene. <i>apr</i> | Michal Letek, unpublished |
| pOK1B | pOKD derivative containing the 5'-end of <i>pbp1b</i> (930 nt) under the control of <i>PgntK. apr</i> | This work |
| pOK2B | pOKD derivative containing the 5'-end of <i>pbp2b</i> (810 nt) under the control of <i>PgntK. apr</i> | This work |
| pNV4A | pOJ260 derivative containing <i>egfp2-ftsI_{CG}</i> under <i>Pkan. apr</i> | (Valbuena <i>et al.</i> , 2006) |
| pOJKG1A, 1B, 2A, 2B serie | pOJ260 derivative containing <i>egfp2-Δpbp1a, 1b, 2a, 2b</i> (respectively) under <i>Pkan</i> and used for integration and expression of the gene fusion. <i>apr</i> | This work |
| Plasmids for Two hybrid system | | |
| pBT | Two-hybrid system bait plasmid containing <i>cat</i> gene, p15A origin of replication and λ cl ORF | Stratagene |
| pTRG | Two-hybrid system target plasmid containing <i>tet</i> gene, ColE1 origin of replication and RNAPα ORF | Stratagene |
| pBT-D | pBT derivative containing a 1.1-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>divIVA_{CG}</i> gene | This work |
| pBT-FtsW | pBT derivative containing a 1.4-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>ftsW</i> gene | This work |
| pBT-FtsZ | pBT derivative containing a 1.3-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>ftsZ</i> gene | This work |
| pBT-RodA | pBT derivative containing a 1.3-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>rodA</i> gene | This work |
| pBT-FtsI | pBT derivative containing a 1.9-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>ftsI</i> gene | This work |
| pBT-1A | pBT derivative containing a 2.4-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>pbp1a</i> gene | This work |
| pBT-1B | pBT derivative containing a 2.2-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>pbp1b</i> gene | This work |
| pBT-2A | pBT derivative containing a 1.9-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>pbp2a</i> gene | This work |
| pBT-2B | pBT derivative containing a 1.5-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>pbp2b</i> gene | This work |

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| pTRG-D | pTRG derivative containing a 1.1-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>divIVA_{CG}</i> gene | This work |
| pTRG-FtsW | pTRG derivative containing a 1.4-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>ftsW</i> gene | This work |
| pTRG-FtsZ | pTRG derivative containing a 1.3-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>ftsZ</i> gene | This work |
| pTRG-RodA | pTRG derivative containing a 1.3-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>rodA</i> gene | This work |
| pTRG-FtsI | pTRG derivative containing a 1.9-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>ftsI</i> gene | This work |
| pTRG-1A | pTRG derivative containing a 2.4-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>pbp1a</i> gene | This work |
| pTRG-1B | pTRG derivative containing a 2.2-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>pbp1b</i> gene | This work |
| pTRG-2A | pTRG derivative containing a 1.9-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>pbp2a</i> gene | This work |
| pTRG-2B | pTRG derivative containing a 1.5-kb fragment from <i>C. glutamicum</i> ATCC 13032 amplified by PCR and carrying the <i>pbp2b</i> gene | This work |

ATCC: American Type Culture Collection. Apr^R and Kan^R are resistances to apramycin and kanamycin respectively. *apr*, *bla* and *kan* are respectively apramycin, ampicillin and kanamycin resistance genes.

Table S2. Primers used in this work

| Experiment | gene | primer |
|--|---------------|---|
| Cloning <i>pbp</i> genes into pGEM-TEasy | <i>pbp1a</i> | 5'-GGAATTCATATGTCCACCACGAATTCTCTGAC-3' (NdeI) |
| | | 5'-CCTCGAGGCCGTGCAGATGGAAAACG-3' (XhoI) |
| | <i>pbp1b</i> | 5'-GGGAATTCATATGACGAATAGTAAAAATCC-3 (NdeI) |
| | | 5'-GTCGACTTAGCCGATCCCTAAGAGAT-3' (Sall) |
| | <i>pbp2a</i> | 5'-GGAATTCATATGAACAAGGTGCAGCGCAGGTC-3' (NdeI) |
| | | 5'-CCTCGAGCTGATTGGGGTAGGAATTCGG-3' (XhoI) |
| | <i>pbp2b</i> | 5'-GGAATTCATATGTGAACCGCTCGATTCAATCACATC-3' (NdeI) |
| | | 5'-CCGCTCGAGTTAAAAATCCTCCGGCTGCCTG-3' (XhoI) |
| Disruption of <i>pbp</i> genes | <i>pbp1a</i> | 5'-GCTCTAGAGATTACGCTCTGCAATATCTTTCTGAGCAGGG-3' (XbaI) |
| | | 5'-CCAAGCTTGGTGTCCACGCCAACCTGCTCGATCATTTTC-3' (HindIII) |
| | <i>pbp1b</i> | 5'-GCTCTAGAGTTCGCTGATCCGTAAGGCTAAGG-3' (XbaI) |
| | | 5'-CCAAGCTTCCACGGGTTTGACATCATCC-3' (HindIII) |
| | <i>pbp2a</i> | 5'-GCTCTAGACTGACCAAGATGCGCAACGAAT-3' (XbaI) |
| | | 5'-CCAAGCTTTGCGAGGATTTACCAGTGGTAGG-3' (HindIII) |
| Placing <i>pbp</i> gene under <i>PgntK</i> | <i>pbp1b</i> | 5'-GGGAATTCATATGACGAATAGTAAAAATCC-3 (NdeI) |
| | | 5'-GGAATTCACGGGTTTGACATCATC-3' (EcoRI) |
| | <i>pbp2b</i> | 5'-GGAATTCATATGTGAACCGCTCGATTCAATCACATC-3' (NdeI) |
| | | 5'-GGAATTCAGGGTAGTGGTGCCACCG-3' (EcoRI) |
| Fusions <i>gfp-pbp</i> | <i>pbp1a</i> | 5'-GGAATTCATATGTCCACCACGAATTCTCTGAC-3' (NdeI) |
| | | 5'-GCGATATCGAAAGCCGAAGCCTCGTCTGG-3' (EcoRV) |
| | <i>pbp1b</i> | 5'-GGGAATTCATATGACGAATAGTAAAAATCC-3 (NdeI) |
| | | 5'-GCGATATCGACATAGTTCCAACGCGTTTCCG-3' (EcoRV) |
| | <i>pbp2a</i> | 5'-GGAATTCATATGAACAAGGTGCAGCGCAGGTC-3' (NdeI) |
| | | 5'-CCAAGCTTTGCGAGGATTTACCAGTGGTAGG-3' (HindIII) |
| | <i>pbp2b</i> | 5'-GGAATTCATATGTGAACCGCTCGATTCAATCACATC-3' (NdeI) |
| | | 5'-GCGATATCAGGGTAGTGGTGCCACCG-3' (EcoRV) |
| Bacterial Two-Hybrid System | <i>divIVA</i> | 5'-GGAATTCATGCCGTTGACTCCA-3' (EcoRI) |
| | | 5'-TATATAGATATCTTACTCACCAGATGGCTTG-3' (EcoRV) |
| | <i>ftsW</i> | 5'-GGAATTCATGACCACCGGAGCC-3' (EcoRI) |
| | | 5'-TATATACTCGAGCTACCAGTCGAGCGT-3' (XhoI) |
| | <i>ftsZ</i> | 5'-GGAATTCATGACCTCACCGAACAA-3' (EcoRI) |
| | | 5'-TATATACTCGAGTTACTGGAGGAAGCT-3' (XhoI) |
| | <i>rodA</i> | 5'-TATATAGCGGCCGCAATGAACACGCTTGAA-3' (NotI) |
| | | 5'-GGAATTCACGCGAGCCACCTCCG-3' (EcoRI) |
| | <i>ftsI</i> | 5'-GGAATTCATGACCTACCGGCCTAAATC-3' (EcoRI) |
| | | 5'-TATATACTCGAGTTATTGAGCTTGAAGGATGA-3' (XhoI) |
| | <i>pbp1a</i> | 5'-TATATAGCGGCCGCGTGTCCACCACGAAT-3' (NotI) |
| | | 5'-TATATACTCGAGCTAGCGGAAGAAGTGGTTGA-3' (XhoI) |
| | <i>pbp2a</i> | 5'-TATATAGCGGCCGCATGAACAAGGTGCAG-3' (NotI) |
| | | 5'-TATATACTCGAGTTAGCCGACTGGCTGCTCTT-3' (XhoI) |
| | <i>pbp2b</i> | 5'-TATATAGCGGCCGCGTGAACCGCTCGATT-3' (NotI) |
| | | 5'-TATATACTCGAGTTAAAAATCCTCCGGCTGCCT-3' (XhoI) |

Table S3. Penicillin Binding Proteins [DD-transpeptidases (TPase), DD-endopeptidases (EPase), DD-carboxypeptidases (CPase), transglycosylases (TGase) and β -lactamases (Blase)] in *Corynebacterium glutamicum*.

| Gene | Chromosome position | Protein | ¹ Amino acids number (in/ membrane/ out) | COG | Putative activities |
|--------------------------------------|---------------------|-----------|---|---------|---------------------|
| <i>pbp1a</i> (NCgl0274) | 294001 | PBP1a | 795 (1-6/ 7-29/ 30-795) | COG0744 | TGase TPase |
| <i>pbp1b</i> (NCgl2884) | 3187040 | PBP1b | 720 (1-24/ 25-47/ 48-720) | COG0744 | TGase TPase |
| ² <i>pbp3</i> (NCgl2084) | 2291209 | PBP3/FtsI | 651 (1-20/ 21-43/ 44-651) | COG0768 | TPase |
| <i>pbp2a</i> (NCgl1933) | 2121293 | PBP2a | 622 (1-7/ 8-25/ 26-622) | COG0768 | TPase |
| ³ <i>pbp2b</i> (NCgl0042) | 43923 | PBP2b | 474 (1-6/ 7-29/ 30-474) | COG0768 | TPase |
| <i>pbp4</i> (NCgl0650) | 695074 | PBP4 | 436 (1-409/ 410-429/ 430-436) | COG1686 | CPase |
| <i>pbp4b</i> (NCgl2606) | 2871443 | PBP4b | 427 (1-4/ 5-27/ 28-427) | COG2027 | EPase CPase |
| <i>pbp5</i> (NCgl2331) | 2559107 | PBP5 | 342 (1-6/ 7-24/ 25-342) | COG1680 | CPase BLase |
| <i>pbp6</i> (NCgl2178) | 2391182 | PBP6 | 275 (no transmembrane helices) | COG1680 | CPase BLase |

¹ The transmembrane helices (in/membrane/out) were obtained from TMHMM Server v.2.0. (<http://www.cbs.dtu.dk/services/TMHMM/>)

² This gene is included in the conserved *dcw* cluster.

³ This gene is included in a conserved cluster comprising the following genes: *ppp*, *rodA*, *pbp2b*, *pknA* and *pknB*.