Expression of the Burkholderia cenocepacia Bep exopolysaccharide is under negative control of the BDSF-dependent quorum sensing system

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Supplementary Figure 1. Colony morphology of *B. cenocepacia* strains on NYG, AB 1.5% glycerol or AB glucose 10mM. Cell suspensions of wild-type *B. cenocepacia* H111 (WT) and mutants carrying an in-frame deletion of *rpfF*, *rpfR* or both genes were spotted on NYG or AB minimal media with 1.5% glycerol or 10mM glucose as the carbon source agar plates. Colony morphology was assayed after growth for 6 and 12 days.
Supplementary Figure. 2. Macrocolony morphology and localized β-galactosidase expression of the bepB::lacZ reporter strains with and without ectopic expression of berA after growth for 12 days on NYG agar plates supplemented with X-gal and appropriate antibiotics.

Supplementary Figure. 3. Influence of growth media on Bep expression. Quantification of β-galactosidase activity (Miller units) of strains harbouring the bepB::lacZ reporter in the wild-type (WT) and rpfR mutant (∆rpfR) background. Reporter strains were grown for 24 h in LB Lennox broth (LB), NYG medium (NYG), AB minimal medium supplemented with 1 % glucose (AB_Glucose), and AB minimal medium supplemented with 1.5 % glycerol (AB_Glycerol).
Supplementary Table 1: Oligonucleotides used in this study

| Name           | Sequence (5'-3') | Restriction site |
|----------------|-----------------|-----------------|
| GmR_F          | ACTGCTCGAGCTAATTCATTAATTGCG | XhoI            |
| GmR_R          | ACTGGTCAACTTCATTAGGTGGCGGTA | SalI           |
| MCS_GPI_F      | AAATTCATGGATATCCATTCGGCTGAGCATGCGGTAC | NcoI           |
| MCS_GPI_R      | CTAGGTAATCGAGCTGACCACATGAGATTCAATCAGATCTG | NcoI           |
| MCS2_GPI_F     | CATGGACTAGTTCTAGACTCGAGCATGCAGGCTGAC | NcoI           |
| MCS2_GPI_R     | CGCATGCTGGATATCGAGACCACCCGCATCCAGAC | NcoI           |
| pQE-rpfR-F     | GGATCCATATGGGATGACGAAACGATAGCGCG | NcoI           |
| pQE-rpfR-R     | AAGCTTACGCGGATCAGGCTGAGCTG | NcoI           |
| P14            | GTAGAATTCGCACTGCTCATCAGGAT | NcoI           |
| P15            | ACTGAGATTCATGGAGACCGCCCGATCCAGAC | NcoI           |
| P16            | ACTGGTAGCATGAGCCCTGACGTCG | NcoI           |
| P20            | TCGAAAGCTTTGATCGGCTGCGTACGCTG | NcoI           |
| P46            | TGACGGTTACCCAGGATGCTAGCGG | NcoI           |
| P47            | AGTCATGCTATAGCTCAGTGAG | NcoI           |
| P48b           | GACGGAATTCGCACTGCTCATGACG | NcoI           |
| P49            | CTGACCATGCGAGCTGACTCGACG | NcoI           |
| P50b           | TGACGGTTACCCAGGATGCTAGCGG | NcoI           |
| P66            | AGTCGAAATTCGGCAGATGATGTGTCG | KpnI           |
| P67            | CAGTCCATGCGCACTTTGCTGACG | KpnI           |
| P68            | AGTCGCAATTCGCACTGCTGACTCG | KpnI           |
| P69            | GTACAGGTATCCCGCATGTCATGTCG | KpnI           |
| P73            | TACGAGATTCGCACTGCTCATGACG | KpnI           |
| P74            | AGTCGCAATTCGCACTGCTCATGACG | KpnI           |
| P75            | AGTCGCAATTCGCACTGCTCATGACG | KpnI           |
| P76            | CTGAGAATTTCGCACTGCTCATGACG | KpnI           |
| P80            | AGTCGGAATTCGCACTGCTCATGACG | KpnI           |
| P81            | GTACGCAATTCGCACTGCTCATGACG | KpnI           |
| P82            | GTACGCAATTCGCACTGCTCATGACG | KpnI           |
| P83            | GTACGCAATTCGCACTGCTCATGACG | KpnI           |
| P86            | TACGAGATTCGCACTGCTCATGACG | KpnI           |
| P87            | ACTGCGATGCGAAGAGGGAG | NcoI           |
| P88            | GTACGCAATTCGCACTGCTCATGACG | NcoI           |
| P89            | CTGAGAATTCGCACTGCTCATGACG | NcoI           |
| P90            | AGTCGCAATTCGCACTGCTCATGACG | KpnI           |
| P91            | GTACGCAATTCGCACTGCTCATGACG | KpnI           |
| P92            | GTACGCAATTCGCACTGCTCATGACG | KpnI           |
| P93            | GTACGCAATTCGCACTGCTCATGACG | KpnI           |
| P94            | GTACGCAATTCGCACTGCTCATGACG | KpnI           |
| P95            | GTACGCAATTCGCACTGCTCATGACG | KpnI           |
| P96            | GTACGCAATTCGCACTGCTCATGACG | KpnI           |
| P103           | ACTGCTAGAACATCCCTGGAGCATGTAAC | XbaI           |
| P104           | ACTGAAAGCTTTATTAAAGAGACCTGCGCAG | XbaI           |
| P120           | ACTGGAATTTCGCAAGAGGACTGTCGGAATG | KpnI           |
| P121           | ACTGCGATGCGAAGAGGGAGAATGATGATG | KpnI           |
| P122           | ACTGCGGATGCGAAGAGGGAGA | KpnI           |
| P214           | AGTCATGCTATAGCGGCAAGAGCATGACG | XbaI           |
| P221           | AGTCGGAATTCGCACTGCTCATGACG | KpnI           |
| P226           | AGTCGGAATTCGCACTGCTCATGACG | KpnI           |
| P237           | ACTGCTAGAACATCCCTGGAGCATGTAAC | XbaI           |
| P238           | ACTGCGGATGCGAAGAGGGAGAATGATGATG | KpnI           |
| P239           | ACTGCGGATGCGAAGAGGGAGAATGATGATG | XbaI           |
| P272           | ACTGCTAGAACATCCCTGGAGCATGTAAC | XbaI           |
| P273           | AGTCGGTACCCACGAGGCG | KpnI           |
## Supplementary Table 2: Plasmids used in this study.

| Plasmid                        | Characteristics                                                                 | Source/Reference |
|--------------------------------|---------------------------------------------------------------------------------|------------------|
| pBluescript SK(+)              | Standard cloning vector                                                          | Stratagene       |
| pGPI-SceI                      | Suicide plasmid vector with I-SceI restriction site, TpR                           | 1                |
| pDAIGm-SceI                    | Plasmid encoding the I-SceI nuclelease gene, GmR                                 | This study       |
| pBBR1MCS-5                     | Broad-host-range cloning vector, GmR                                              | 2                |
| pYhck                          | pRK404A carrying the *E. coli* yedQ (yhck) gene, TeR                            | 3                |
| pYedQ                          | pBBR1MCS-5 carrying the *E. coli* yedQ (yhck) gene, GmR                        | This study       |
| pPA5295                        | Pseudomonas aeruginosa PDE on pBBR1MCS-5, GmR                                    | 4                |
| pBBR-rpfR                      | pBBR1MCS carrying H111 wild-type rpfR, CmR                                      | 5                |
| pBBR-rpfRGGAAF                 | pBBR-rpfR harboring D318A and E319A amino acid substitutions, CmR               | 6                |
| pBBR-rpfRAAL                   | pBBR-rpfR harboring an E443A amino acid substitution, CmR                        | 6                |
| pRpfR                         | pBBR1MCS-5 carrying the berA gene, GmR                                          | 7                |
| pBerA                          | pBBR1MCS-5 carrying the berA gene, GmR                                          | This study       |
| pRpoN                          | pBBR1MCS-5 carrying the bc0813 gene, GmR                                        | This study       |
| pBerB                          | pBBR1MCS-5 carrying the berB gene, GmR                                          | This study       |
| pQE-32                         | Expression vector for 6xHis-tagged proteins                                      | Qiagen           |
| pQE-RpfR                      | Expression vector for 6xHis-tagged RpfR                                          | This study       |
| pQE-RpfRGGAAF                  | Expression vector for 6xHis-tagged RpfRGGAAF                                     | This study       |
| pQE-RpfRAAL                    | Expression vector for 6xHis-tagged RpfRAAL                                       | This study       |
| pGPI-ArpR                      | pGPI-SceI based deletion plasmid for rpfR, TpR                                  | This study       |
| pGPI-ΔrpfF                     | pGPI-SceI based deletion plasmid for rpfF, TpR                                  | This study       |
| pGPI-ΔrpfFR                    | pGPI-SceI based deletion plasmid for rpfR and rpfF, TpR                        | This study       |
| pGPI-ΔrpfOc                    | pGPI-SceI based deletion plasmid for rpoN, TpR                                  | This study       |
| pGPI-AcbeC                     | pGPI-SceI based deletion plasmid for bceC, TpR                                  | This study       |
| pGPI-ΔgtaB                     | pGPI-SceI based deletion plasmid for gtaB, TpR                                  | This study       |
| pGPI-AcbeB                     | pGPI-SceI based deletion plasmid for bepB, TpR                                  | This study       |
| pGPI-ΔberA                     | pGPI-SceI based deletion plasmid for berA, TpR                                  | This study       |
| pGPI-beP-B::lacZ               | Plasmid for insertion of lacZ downstream of bepB, TpR                          | This study       |
| pSUP3535                       | transcriptional lacZ fusion vector, TeR                                         | 8                |
| pGPI2-SceI                     | pGPI-SceI with modified multiple cloning site, TpR                              | This study       |
| pGPI2-rpfRGGAAF                | pGPI-SceI based knock-in plasmid for rpfRGGAAF, TpR                            | This study       |
| pGPI2-rpfRAAL                  | pGPI-SceI based knock-in plasmid for rpfRAAL, TpR                             | This study       |
| pGPI2-ΔrpfWT                   | pGPI-SceI based knock-in plasmid for rpfRWW, TpR                               | This study       |
| pUT18-BerB                     | Euromedex plasmid with BerB-adenylate cyclase domain 18, Ap100                  | This study       |
| pUT18C-BerB                    | Euromedex plasmid with adenylate cyclase domain 18-BerB, Ap100                 | This study       |
| pKKT25-RpfR                    | Euromedex plasmid with adenylate cyclase domain 25 -BerB, Kn50                  | This study       |
| pKNT25-RpfR                    | Euromedex plasmid with RpfR- adenylate cyclase domain 25, Kn50                  | This study       |

Gm, gentamycin; Tc, tetracycline, Tp, trimethoprim, Ap, ampicillin, Kn, kanamycin
Supplementary References

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