**Supplement**

**Na+ homeostasis in *Acinetobacter baumannii* is facilitated**

***via* the activity of the** **Mrp antiporter**

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Running title: Na+ cycling in *A. baumannii* ATCC 19606

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**Tab. S1 Genes encoding Mrp antiporter subunits in *A. baumannii* ATCC 19606 & their properties**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Locus tag | Encoded protein | Gene length (bp) | Molecular mass [kDa] | Predicted transmembrane helices\* |
| *F911\_03564* | MrpA’ | 2835 | 102.5 | 25 |
| *F911\_03565* | MrpC | 366 | 13.1 | 3 |
| *F911\_03566* | MrpD | 1809 | 66.8 | 14 |
| *F911\_03567* | MrpE | 525 | 20.0 | 4 |
| *F911\_03568* | MrpF | 273 | 9.9 | 3 |
| *F911\_03569* | MrpG | 402 | 14.9 | 3 |

 \* Prediction of transmembrane helices was done by the program Expasy



**Fig. S1** Genetic organization of the *mrp* gene cluster of *A. baumannii* ATCC 19606 and the *A. baumannii* ATCC 19606 ∆*mrpA’* mutant.

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**Fig. S2** Genetic complementation of *A. baumannii* ATCC 19606 ∆*mrpA*. Growth of *A. baumannii* ATCC 19606\_F911\_00233::*kanR*(A), ∆*mrpA’*\_F911\_00233::*kanR* (B) ∆*mrpA’*\_*F911\_00233*:: *mrpA’*\_*kanR* (C) was monitored in L0-medium (○) with 5 mM LiCl (□), 300 mM NaCl (△) or an initial pH of 10 (▽) in the presence of 20µg/ ml kanamycin. Precultures were grown in L0-medium (pH 7) overnight in the presence of 20 µg/ ml kanamycin and were used to inoculate prewarmed medium to an initial OD600nm of 0.1 Error bars denote the standard deviation from at least three biological replicates.



**Fig. S3** Activity of the *mtlD* promoter determined by reporter gene assays. (A) Growth and (B) corresponding β-glucoronidase activity of the reporter gene strain ATCC 19606 + pVRL2\_up\_*mtlD*\_*gusA* grownin mineral medium (○) with 200 mM NaCl (□). Error bars denote standard deviation calculated from at least three biological replicates.



**Fig. S4** Desiccation resistance of *A. baumannii* ATCC 19606\_*F911\_00233*::*kanR* (△), ∆*mrpA’*\_*F911\_00233*::*kanR* (□) ∆*mrpA’*\_*F911\_00233*::*mrpA’*\_*kanR* (○). *A. baumannii* strains were spotted on culture plates and incubated in a climate chamber at 22°C and 31% relative humidity (RH). Bacteria were removed from the plates by resuspending with 360 µl K+-phosphate buffer (10 mM, pH 6.8). Appropriate dilutions were prepared and cell forming units were determined on solid L0-medium. Error bars denote the standard deviation calculated from at least three biological replicates.



**Fig. S5** Growth of *A. baumannii* wild type and ∆*mrpA’* in inactivated human urine. *A. baumannii* ATCC 19606, ∆*mrpA’*, ATCC 19606\_*F911\_00233*::*kanR*, ∆*mrpA’*\_F911\_00233::*kanR* and ∆*mrpA’*\_F911\_00233::*mrpA’*\_*kanR* were grown in L0-medium overnight in the presence of 20 µg/ml kanamycin. Precultures were used to inoculate prewarmed inactivated human serum to an initial OD600 of 0.1. Cells were incubated for 24 at 37 °C and the optical density was measured.

**Tab S2** Primers used in this study.

|  |  |  |
| --- | --- | --- |
| pBIISK\_*sacB*\_*kanR*\_fwd | GGCTGCAGGAATTCGATG | Construction of pBIISK\_*sacB*\_*kanR*\_∆*mrpA* |
| pBIISK\_*sacB*\_*kanR*\_rev | CGGGGGATCCACTAGTTCTAGAGCGG | Construction of pBIISK\_*sacB*\_*kanR*\_∆*mrpA* |
| *mrpA*\_up\_fw | AGCATCGAATTCCTGCAGCCTTCCACCGGAATTTGGAC | Construction of pBIISK\_*sacB*\_*kanR*\_∆*mrpA* |
| *mrpA*\_up\_rev | TCGAGTGGCGACAAGGGTGGTGCCTAATATTAAC | Construction of pBIISK\_*sacB*\_*kanR*\_∆*mrpA* |
| *mrpA*\_down\_fwd | CCACCCTTGTCGCCACTCGAGTATGTCTG | Construction of pBIISK\_*sacB*\_*kanR*\_∆*mrpA* |
| *mrpA*\_down\_rev | TAGAACTAGTGGATCCCCCGAACAGGTAAAATGCTGCAC | Construction of pBIISK\_*sacB*\_*kanR*\_∆*mrpA* |
| Mrp fw | GGAAGCCGTGAAGAATTG | Control primer  |
| Mrp rev | TTTATTCTTGAGGAGGTTCC | Control primer |
| pBIISK\_fwd  | AGTGGATCCCCCGGGCTG  | Construction of pBIISK\_∆*mrpA*’::*kanR* |
| pBIISK\_rev  | AGTTCTAGAGCGGCCGCC  | Construction of pBIISK\_∆*mrpA*’::*kanR* |
| Up\_*mrpA*\_fwd  | GTGGCGGCCGCTCTAGAACTGCTCAATTTTTATAATAAAACCG  | Construction of pBIISK\_∆*mrpA*’::*kanR* |
| Up\_*mrpA*\_rev  | CACAATCGCTCATATATAATTTAGACCGAAGCC  | Construction of pBIISK\_∆*mrpA*’::*kanR* |
| *kanR*\_*mrpA*\_fwd  | ATTATATATGAGCGATTGTGTAGGCTGG  | Construction of pBIISK\_∆*mrpA*’::*kanR* |
| *kanR*\_*mrpA*\_rev  | AAACTGATCAATATGAATATCCTCCTTAGTTCCTATTC  | Construction of pBIISK\_∆*mrpA*’::*kanR* |
| Down\_*mrpA*\_fwd  | ATATTCATATTGATCAGTTTAGAATTCTTACTG  | Construction of pBIISK\_∆*mrpA*’::*kanR* |
| Down\_*mrpA*\_rev  | TGCAGCCCGGGGGATCCACTCGGATTCATAACGGCTAC  | Construction of pBIISK\_∆*mrpA*’::*kanR* |
| LPP\_fw | GTTGCGCAATACAACTTG | Amplification PCR product  |
| LPP\_rev | AGTTAACCGCATAGCCCAGC | Amplification PCR product  |
| pBIISK\_fw | ATCGAATTCCTGCAGCCC | Construction of pBIISK\_*mrpA’&* pBIISK\_*kanR* |
| pBIISK\_rev | ATCAAGCTTATCGATACCGTC | Construction of pBIISK\_*mrpA’ &* pBIISK\_*kanR* |
| Up\_fw | ACGGTATCGATAAGCTTGATGAAGCCTACCTCAAGATAAG | Construction of pBIISK\_*mrpA’&* pBIISK\_*kanR* |
| Up\_rev | CACAATCGCTGCTAAGAGGTTTCACTTC | Construction of pBIISK\_*mrpA’&* pBIISK\_*kanR* |
| *KanR*\_fw | ACCTCTTAGCAGCGATTGTGTAGGCTGG | Construction of pBIISK\_*mrpA’* |
| *KanR*\_rev | GGAGCTGTCCATATGAATATCCTCCTTAGTTCCTATTC | Construction of pBIISK\_*mrpA’* |
| *KanR*\_rev | TACCATCAGCATATGAATATCCTCCTTAGTTCCTATTC | Construction of pBIISK\_*kanR* |
| *mrpA*\_fw | ATATTCATATGGACAGCTCCCTGAAACC | Construction of pBIISK\_*mrpA’* |
| *mrpA*\_rev | TACCATCAGCTCATGATGATTTCTCCCCATG | Construction of pBIISK\_*mrpA’* |
| down\_fw | ATCATCATGAGCTGATGGTAGTGTGGGATTAC | Construction of pBIISK\_*mrpA’* |
| down\_fw | ATATTCATATGCTGATGGTAGTGTGGGATTAC | Construction of pBIISK\_*kanR* |
| down\_rev | CCGGGCTGCAGGAATTCGATAAGGATCTGCAGGAATCAATTC | Construction of pBIISK\_*kanR* & pBIISK\_*mrpA’* |
| LPP\_fw | TAAGATTCAATCAGCCCAGTC | amplification PCR-product |
| LPP\_rev | ATAGGTACTTACCCCCTTATTC | amplification PCR-product |
| Check-fw | ATCGCCGGGTAGCTATGTTC | Control primer |
| Check-rev | TCTTTCTGGCGGTAATCC | Control primer |
| pVRL2\_up fw | GAGCTCCAATTCGCCCTATAG | Construction of pVRL2\_*upmrpA’*\_*gusA* |
| pVRL2\_up rev | TCTTTCCAGTCGGGAAAC | Construction of pVRL2\_*upmrpA’*\_*gusA* |
| *gusA*\_fw | GGATACGAGTATGGGCAGCAGCCATCAC | Construction of pVRL2\_*upmrpA’*\_*gusA* |
| *gusA*\_rev | TATAGGGCGAATTGGAGCTCCTACCGGCCGCATAGGCC | Construction of pVRL2\_*upmrpA’*\_*gusA* |
| *mrp* up\_fwd gus | AGGTTTCCCGACTGGAAAGAGTTAATTGACCAGATCGATTC | Construction of pVRL2\_*upmrpA’*\_*gusA* |
| *mrp* up\_rev gus | TGCTGCCCATACTCGTATCCATATATAATTTAGAC | Construction of pVRL2\_*upmrpA’*\_*gusA* |
| pVRL2\_*gusA* fw | ATGGGCAGCAGCCATCAC | Construction of pVRL2\_*upmtlD*\_*gusA* |
| pVRL2\_*gusA* rev | TCTTTCCAGTCGGGAAACC | Construction of pVRL2\_*upmtlD*\_*gusA* |
| upstream\_*mtlD* fw | AGGTTTCCCGACTGGAAAGATGCCGAAATTTATGAAGG | Construction of pVRL2\_*upmtlD*\_*gusA* |
| upstream\_*mtlD* rev | TGGTGATGGCTGCTGCCCATTTTTTACCTCTTTTTTGATCATG | Construction of pVRL2\_*upmtlD*\_*gusA* |