Supplementary Figure 1: Principal component analysis (PCA) of the RNA sequencing output. The PCA plot for the variance-stabilized transformation of the DESeq2 object was calculated on the basis of 1,000 top genes. We removed one replicate of 2003.9LB DESeq2 analysis because visualization revealed a shift on PC1 and PC2.
Supplementary Figure 2: Heatmap of differentially expressed genes (n = 459). Most important genes are shown on the left. The regularized log transformation function (rlog) from DESeq2 was used to convert counts to the log2 scale for better visualization.
Supplementary Figure 3: Venn diagram of differentially expressed genes in 2003.2LB, 2003.2MH-2, 2003.9LB, and 2003.9MH-2. The Venn diagram shows the number of upregulated (black) and downregulated (white) genes in the four adapted variants. The visualization was created using ggvenn v.0.1.9.
Supplementary Figure 4: Summary of the results of differential gene expression analysis of 2003.2, 2003.2\textsuperscript{LB}, and 2003.2\textsuperscript{MH-2}. a,b Functional classification of differentially expressed genes of 2003.2\textsuperscript{LB} (a) and 2003.2\textsuperscript{MH-2} (b) each compared to 2003.2 based on the Clusters of Orthologous Groups (COG) database. c Correlation analysis between 2003.2\textsuperscript{LB} (y-axis) and 2003.2\textsuperscript{MH-2} (x-axis). Similar gene expression profiles in the upper-right and lower-left corners indicate transcriptomic changes independent of the chosen broth medium. The most important genes are highlighted and classified according to COG.
Supplementary Figure 5: Summary of the results of differential gene expression analysis of 2003.9, 2003.9LB, and 2003.9MH-2. a,b Functional classification of differentially expressed genes of 2003.9LB (a) and 2003.9MH-2 (b) each compared to 2003.9 based on the Clusters of Orthologous Groups (COG) database. c Correlation analysis between 2003.9LB (y-axis) and 2003.9MH-2 (x-axis). Similar gene expression profiles in the upper-right and lower-left corners indicate transcriptomic changes independent of the chosen broth medium. The most important genes are highlighted and classified according to COG.
**Supplementary Table 1: Results of phenotypic antimicrobial susceptibility testing.**

Interpretive categories according to EUCAST (The European Committee on Antimicrobial Susceptibility Testing 2021: Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0.).

For susceptibility testing purposes, the concentration of the β-lactamase inhibitor is fixed at 4 mg/L.

Ceftazidime:aztreonam in the ratio 1:1 was used. MIC: minimum inhibitory concentration; n.a.: not applicable; R: resistant; S: susceptible.

| Antimicrobials                  | PBIO2003 (wild-type) | 2003.2 | 2003.2\(^{2}\)* | 2003.3\(^{2}\)* | 2003.9 | 2003.9\(^{2}\)* | 2003.9\(^{2}\)* |
|--------------------------------|----------------------|--------|-----------------|-----------------|--------|-----------------|-----------------|
|                                | MIC [µg/mL]          | S/R*   | MIC [µg/mL]     | S/R*            | MIC [µg/mL] | S/R*            | MIC [µg/mL] | S/R* |
| Ampicillin                     | > 32                 | R      | > 32            | R               | > 32     | R               | > 32           | R    |
| Ampicillin-sulbactam\(^{b}\)   | > 32                 | R      | > 32            | R               | > 32     | R               | > 32           | R    |
| Piperacillin                   | > 128                | R      | > 128           | R               | > 128    | R               | > 128          | R    |
| Piperacillin-tazobactam\(^{a}\)| > 128                | R      | > 128           | R               | > 128    | R               | > 128          | R    |
| Cefuroxime                     | > 64                 | R      | > 64            | R               | > 64     | R               | > 64           | R    |
| Cefotaxime                     | > 64                 | R      | > 64            | R               | > 64     | R               | > 64           | R    |
| Cefpodoxime                    | > 8                  | R      | > 8             | R               | > 8      | R               | > 8            | R    |
| Cefazidime                     | > 64                 | R      | > 64            | R               | > 64     | R               | > 64           | R    |
| Ceftriaxime-avibactam\(^{b}\)  | 0.5                  | R      | > 16            | R               | > 16     | R               | > 16           | R    |
| Aztreonam                      | > 128                | R      | > 128           | R               | > 128    | R               | > 128          | R    |
| Ceftriaxime-avibactam:aztreonam\(^{b}\) | > 0.0625/0.0625 n.a. | > 64/64 n.a. | > 64/64 n.a. | > 64/64 n.a. | > 64/64 n.a. | > 64/64 n.a. |
| Ertapenem                      | > 8                  | R      | > 8             | R               | > 8      | R               | > 8            | R    |
| Imipenem                       | > 16                 | R      | > 16            | R               | > 16     | R               | > 16           | R    |
| Meropenem                      | > 16                 | R      | > 16            | R               | > 16     | R               | > 16           | R    |
| Ciprofloxacin                  | > 4                  | R      | > 4             | R               | > 4      | R               | > 4            | R    |
| Moxifloxacin                   | > 8                  | R      | > 8             | R               | > 8      | R               | > 8            | R    |
| Gentamicin                     | > 16                 | R      | > 16            | R               | > 16     | R               | > 16           | R    |
| Trimethoprim-sulfamethoxazole   | ≤ 20                 | S      | ≤ 20            | S               | ≤ 20     | S               | ≤ 20           | S    |
| Tigecycline                    | 2                    | R      | 2               | R               | 2        | R               | 2              | R    |
| Colistin                       | 16                   | R      | 16              | R               | 0.5      | S               | 16             | R    |
| Chloramphenicol                | 8                    | S      | 8               | S               | 8        | S               | 8              | S    |