# Constitutive phenotypic modification of lipid A in clinical *Acinetobacter baumannii* isolates

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**Keywords:** Carbapenem-resistant; Polymyxin; Lipopolysaccharide; Phosphoethanolamine; *pmrC*; Gram-negative bacteria

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**Supplementary Table 1. Primers used in this study.**

| MLST primers | Sequence (5′–3′) | Reference |
|---|---|---|
| *gltA*_F | AATTTACAGTGGCACATTAGGTCCC | (1) |
| *gltA*_R | GCAGAGATACCAGCAGAGATACACG | (1) |
| *gyrB*_F | TGAAGGCGGCTTATCTGAGT | (1) |
| *gyrB*_R | GCTGGGTCTTTTTCCTGACA | (1) |
| *gdhB*_amp_F | GCTACTTTTATGCAACAGAGCC | (1) |
| *gdhB*_amp_R | GTTGAGTTGGCGTATGTTGTGC | (1) |
| *gdhB*_seq_F | ACCACATGCTTTGTTATG | (1) |
| *gdhB*_seq_R | GTTGGCGTATGTTGTGC | (1) |
| *recA*_F | CCTGAATCTTCYGGTAAAAC | (1) |
| *recA*_R | GTTTCTGGGCTGCCAAACATTAC | (1) |
| *cpn60*_F | GGTGCTCAACTTGTTCGTGA | (1) |
| *cpn60*_R | CACCGAAACCAGGAGCTTTA | (1) |
| *gpi*_F | GAAATTTCCGGAGCTCACAA | (1) |
| *gpi*_R | TCAGGAGCAATACCCCACTC | (1) |
| *rpoD*_F | ACCCGTGAAGGTGAAATCAG | (1) |
| *rpoD*_R | TTCAGCTGGAGCTTTAGCAAT | (1) |
| **qRT primers** | **Sequence (5′–3′)** | **Reference** |
| qRT_16s_F(80) | TGGTGCCTTCGGGAATCTAG | This study |
| qRT_16s_R(80) | TGCGGGACTTAACCCAACAT | This study |
| RT_*pmrC*_F(100) | TGGGTAGTCATGGACCTGCAT | This study |
| RT_*pmrC*_R(100) | GTTTGCGAACAGCCCTGTATC | This study |
| RT_*lpxC*_ F(105) | GGTGGCCTGTGTGAACAAGA | This study |
| RT_*lpxC*_ R(105) | GCCATTATGCGGGCTGAATA | This study |
| RT_*lpxK*_ F(100) | TGACGACCTAATGCCCAATG | This study |
| RT_*lpxK*_ R(100) | GACCCAATAGACAAGCTGCAATC | This study |
| RT_*lpxL*_ F(100) | ATGCACAAGCTCAAGGCAAAG | This study |
| RT_*lpxL*_ R(100) | ACATCCGGCTCGAAATATTGG | This study |
| RT_*lpxM*_ F(108) | TCCGGCAAAATTACTGTCGTT | This study |
| RT_*lpxM*_ R(108) | AGAAGCCCGTAGCCGTGAA | This study |
| RT_*lptC*_ F(105) | CGCCTTGTGGAGACTGAACTG | This study |
| RT_*lptC*_ R(105) | CACCGCAAGGAACCATTGA | This study |

| ***pmrCAB* operon primers** | **Sequence (5′–3′)** | **Reference** |
|---|---|---|
| pmr_1_F | TGGTAAGTCTTACAAAGATAACCGTGTTGGT | This study |
| pmr_1_R | GGTTTGCGAACAGCCCTGTATC | This study |
| pmr_2_F | AGATGGCGAATGTTATGATGACATTCTC | This study |
| pmr_2_R | CTTCTTGAAGTGCAACCTTATAAGCAC | This study |
| pmr_3_F | AGCGAAGCTGGGTAAAGATTTTATCC | This study |
| pmr_3_R | GGAGCACATTTCCTAAGCCATAAC | This study |

**Supplementary Table 2. Antibiotic susceptibility tests of clinical *A. baumannii* isolates used in this study.** The minimum inhibitory concentrations of four classes of antibiotics were measured using the two-fold dilution method. *, MIC of polymyxin B in a previous study (2).

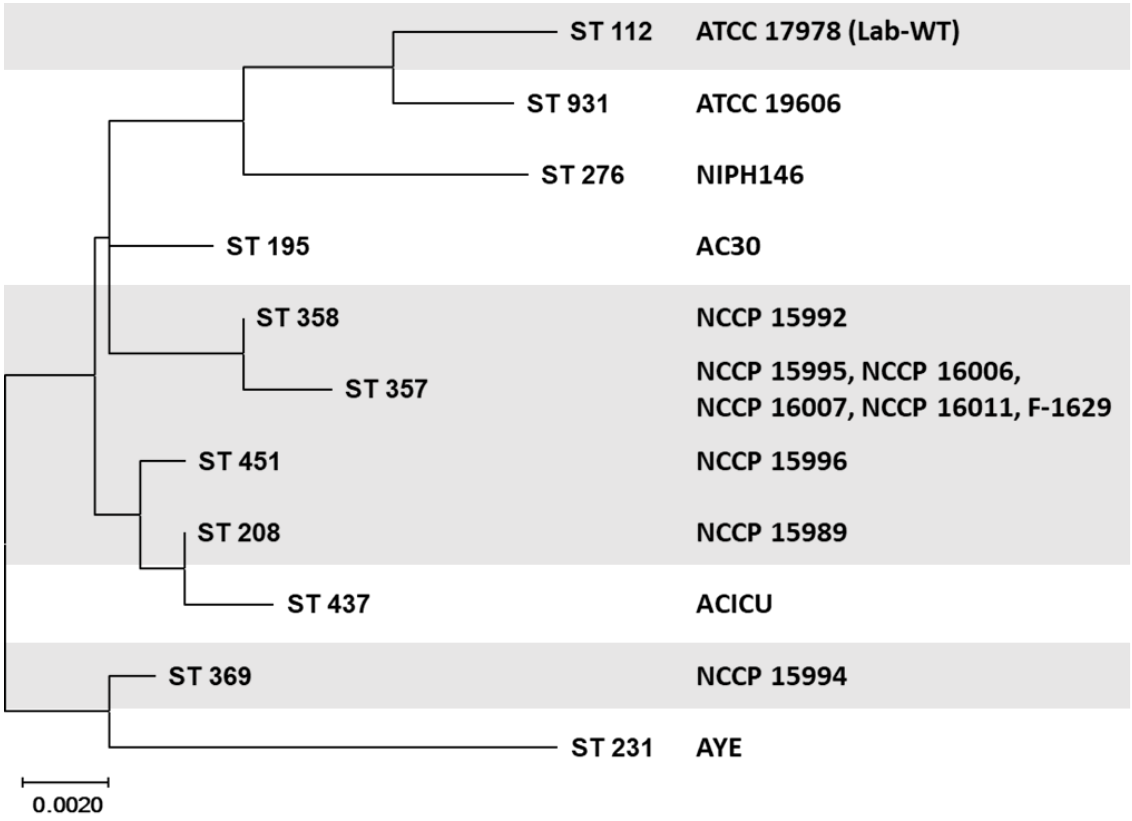
| Strain | Origin | Year | Region | Antibiotics | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Polymyxin B* | Meropenem | Doxycycline | Gentamicin | Erythromycin |
| NCCP 12276 | Other | N/A | Busan | 3 | 2 | 16 | >512 | >512 |
| NCCP 12277 | Pus | N/A | Gwangju | 1.5 | ≤0.5 | 16 | >512 | 512 |
| NCCP 12278 | Sputum | N/A | Gyeongsangnam-do | 2.5 | 16 | 0.5 | 32 | 32 |
| NCCP 14606 | Cerebrospinal fluid | 2005 | Daegu | 2 | ≤0.5 | ≤0.5 | 2 | 4 |
| NCCP 14607 | Sputum | 2005 | Daegu | 1 | >64 | 32 | >512 | 64 |
| NCCP 14608 | Pus | 2004 | Daegu | 2 | 1 | 16 | >512 | >512 |
| NCCP 14609 | Pleural fluid | 2009 | Daegu | 1 | >64 | 1 | >512 | >512 |
| NCCP 14654 | Other | 2009 | Gyeongsangnam-do | 2 | 64 | 2 | >512 | >512 |
| NCCP 14655 | Pus | 2009 | Gyeongsangnam-do | 2 | ≤0.5 | 8 | 512 | 16 |
| NCCP 14782 | Pus | 2009 | Gyeongsangnam-do | 2 | ≤0.5 | ≤0.5 | 2 | 8 |
| NCCP 15987 | Pus | 2013 | Incheon | 2 | ≤0.5 | ≤0.5 | 2 | 16 |
| NCCP 15988 | Sputum | 2013 | Jeollanam-do | 3 | 64 | ≤0.5 | 8 | 32 |
| NCCP 15990 | Sputum | 2013 | Gwangju | 1.5 | >64 | 1 | >512 | >512 |
| NCCP 15991 | Urine | 2013 | Gyeongsangbuk-do | 2 | 64 | 32 | >512 | 512 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| NCCP 15993 | Pus | 2013 | Seoul | 1 | 32 | 32 | 4 | 16 |
| NCCP 15997 | Urine | 2013 | Jeollanam-do | 3 | 32 | ≤0.5 | >512 | >512 |
| NCCP 15998 | Sputum | 2013 | Gyrongsangbuk-do | 2 | 16 | 16 | >512 | 32 |
| NCCP 15999 | Sputum | 2013 | Gyeongsangnam-do | 2.5 | >64 | ≤0.5 | >512 | 64 |
| NCCP 16000 | Prostate | 2013 | Gyeonggi-do | 1.5 | 64 | ≤0.5 | 512 | 16 |
| NCCP 16001 | Other | 2013 | Chungcheongbuk-do | 1.5 | ≤0.5 | ≤0.5 | 2 | 16 |
| NCCP 16002 | Sputum | 2013 | Gangwon-do | 1.5 | 32 | 16 | 2 | 32 |
| NCCP 16003 | Sputum | 2013 | Gyeongsangnam-do | 4 | ≤0.5 | ≤0.5 | 2 | 8 |
| NCCP 16004 | Urine | 2013 | Seoul | 1 | ≤0.5 | ≤0.5 | 1 | 8 |
| NCCP 16005 | Sputum | 2013 | Gyeongsangnam-do | 1.5 | ≤0.5 | ≤0.5 | 2 | 8 |
| NCCP 16008 | N/A | 2011 | Seoul | 2 | 64 | 32 | >512 | 512 |
| NCCP 16009 | N/A | 2011 | Seoul | 1 | 32 | 32 | >512 | 512 |
| NCCP 16010 | N/A | 2011 | Seoul | 2 | 64 | 32 | >512 | 512 |
| F-1025 | N/A | N/A | Seoul | 4 | 64 | 1 | >512 | >512 |
| F-1208 | N/A | N/A | Seoul | 4 | 64 | 32 | >512 | 512 |
| F-1379 | N/A | N/A | Seoul | 2 | ≤0.5 | 16 | >512 | 64 |
| F-1410 | N/A | N/A | Seoul | 2 | 2 | 32 | >512 | >512 |

**Supplementary Table 3. Amino acid variations in PmrCAB in MDR clinical *A. baumannii* isolates.** Common variations in *pmrC* and point mutations of distinct regions in *pmrB* were detected by genomic analysis of the *pmrCAB* operon in comparison with the reference strain (Lab-WT). $PMB^R$, polymyxin B-resistant strains; $PMB^S$, polymyxin B-susceptible strains; ▲, mutation site; △, deletion site.

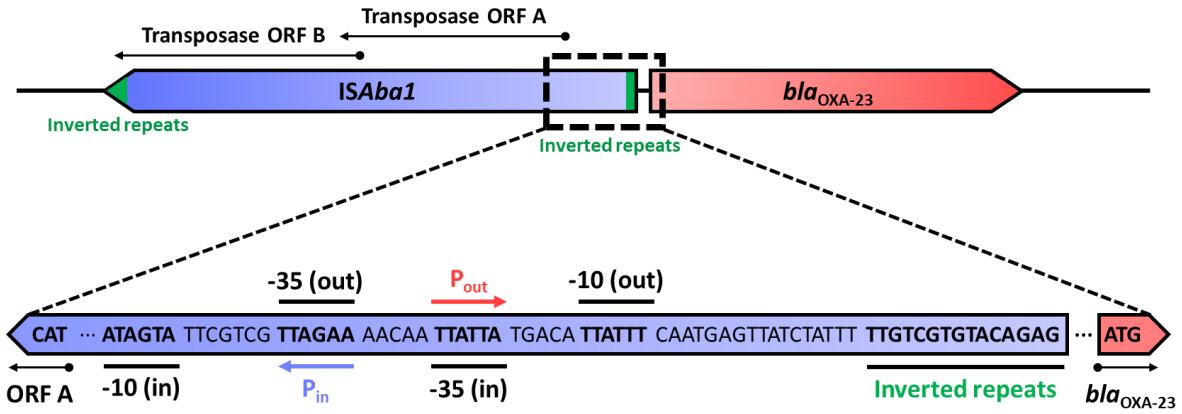
| Category | Isolates | PMB MIC (μg/mL) | Amino acid position | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *pmrC* | | | | | *pmrA* | *pmrB* | | | | | | | | | |
| | | | 150 | 212 | 332 | 354 | 515 | - | 26 | 27–30 | 61 | 138 | 163 | 170 | 233 | 376–399 | 400–444 | 444 |
| **Reference strain** | ATCC 17978 (Lab-WT) | 2 | F | I | R | A | K | - | F | - | S | A | I | P | P | - | - | A |
| **$PMB^R$** | NCCP 16007 | 256 | L | V | K | S | T | - | L | △ | - | - | - | - | - | - | - | V |
| | NCCP 15996 | 256 | L | V | K | S | T | - | - | - | - | T | - | - | - | - | - | V |
| | NCCP 15995 | 128 | L | V | K | S | T | - | - | - | G | - | N | - | - | - | - | V |
| | F-1629 | 128 | L | V | K | S | T | - | - | - | - | - | - | L | - | - | - | V |
| **$PMB^S$** | NCCP 15989 | 2.5 | L | V | K | S | T | - | - | - | - | - | - | - | - | - | - | V |
| | NCCP 16006 | 2 | L | V | K | S | T | - | - | - | - | - | - | - | - | - | - | V |
| | NCCP 16011 | 2 | L | V | K | S | T | - | - | - | - | - | - | - | S | ▲ | △ | △ |
| | NCCP 15992 | 1 | L | V | K | S | T | - | - | - | - | - | - | - | - | - | - | V |
| | NCCP 15994 | 1 | L | V | K | S | T | - | - | - | - | - | - | - | - | - | - | V |

Supplementary Figure 1.



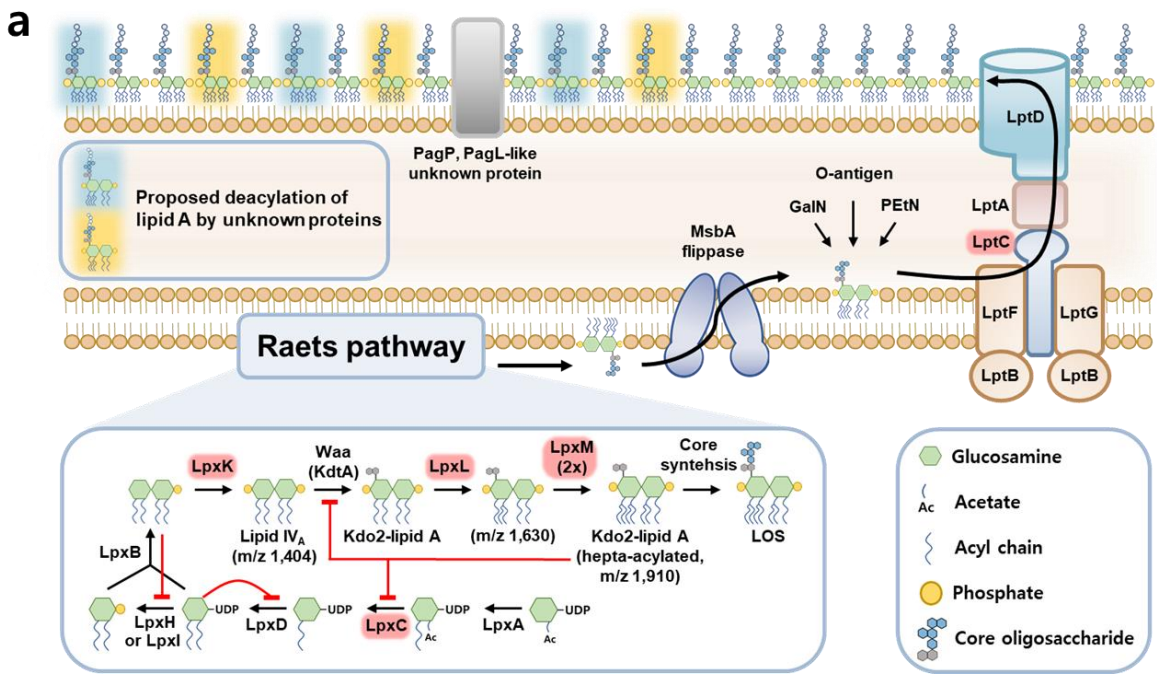

**Supplementary Figure 1. Phylogenetic tree based on the seven housekeeping genes used in multilocus sequence typing analysis.** The sequences of seven genes (*gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD*) used for multilocus sequence typing were combined to create a phylogenetic tree using the MEGA X program. For the phylogenetic tree, ATCC 19606, NIPH146, AC30, ACICU, and AYE were representative strains for ST931, ST276, ST195, ST437, and ST231, respectively. The gray background denotes strains used in this study.

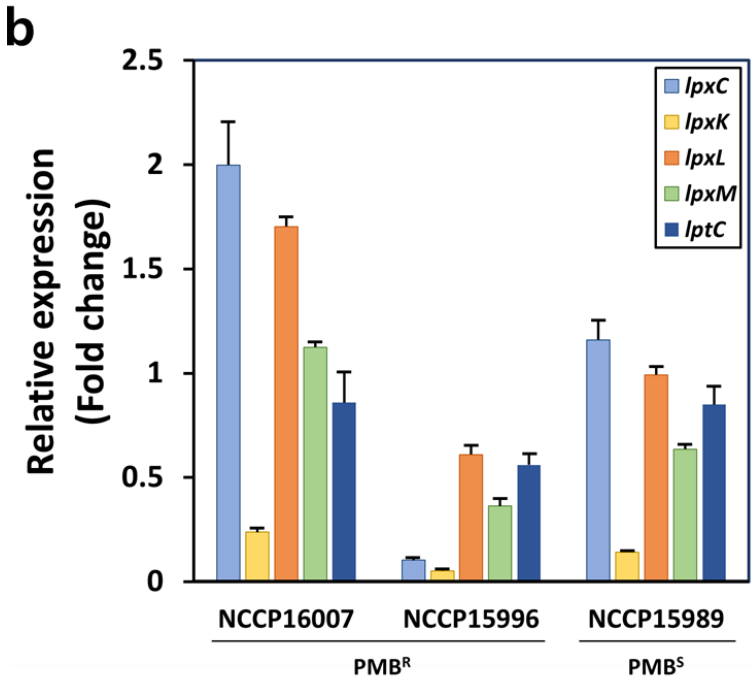
Supplementary Figure 2.




**Supplementary Figure 2. Genetic schematic diagram of IS*Aba1*/*bla*$_{OXA-23}$.** The mobile genetic element IS*Aba1* contains two inverted repeat sequences and two transposase open-reading frames. The transposase inside IS*Aba1* is controlled by the $P_{in}$ promoter, and the $P_{out}$ promoter regulates the anti-sense gene in the region where IS*Aba1* is inserted. The *bla*$_{OXA-23}$ antibiotic resistance gene is regulated by the $P_{out}$ promoter located upstream of IS*Aba1*, resulting in carbapenemase production.

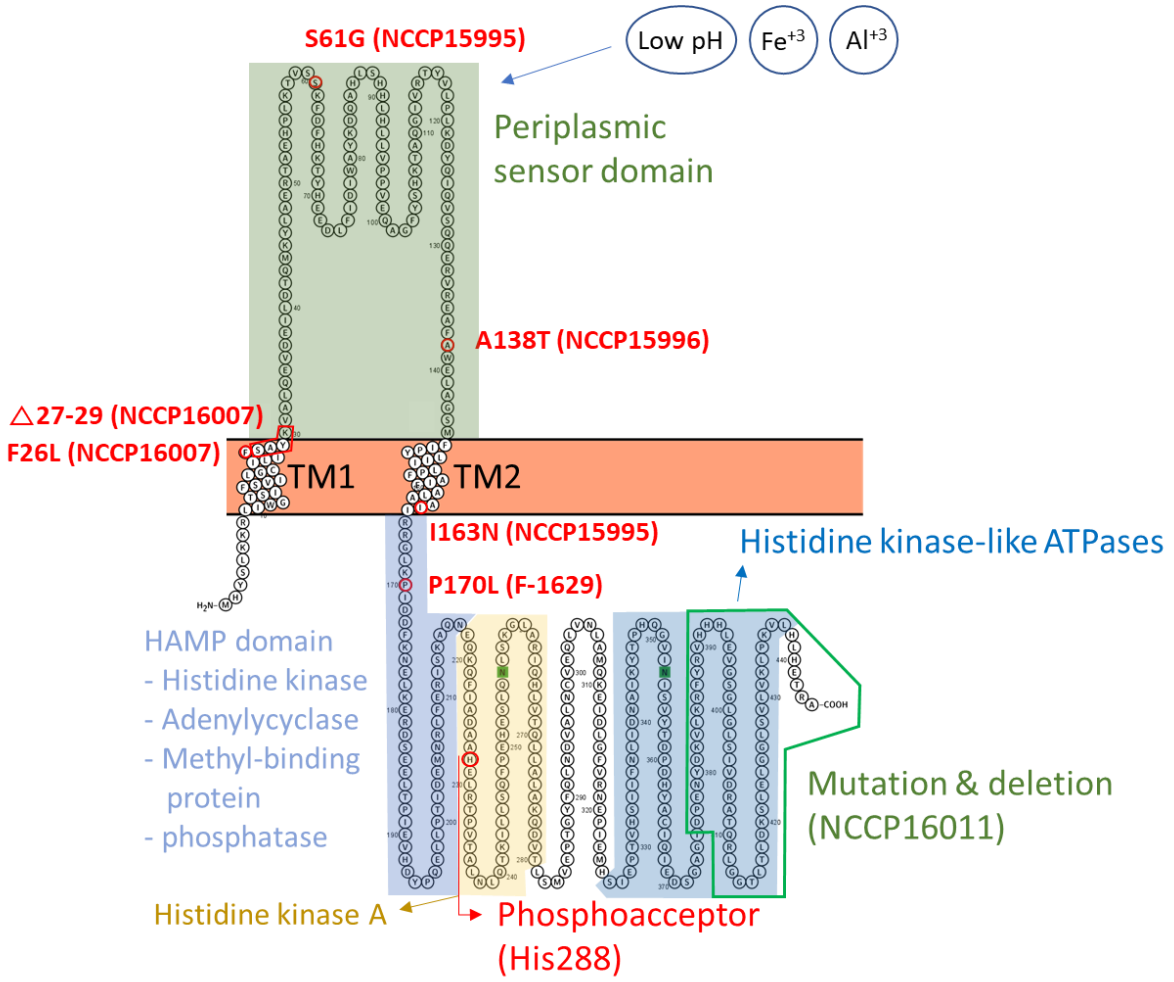
Supplementary Figure 3.



**Supplementary Figure 3. Lipid A biosynthetic pathway.** (a) Proposed biosynthesis and transport of *A. baumannii* lipooligosaccharide. Feedback inhibition is indicated by a red line. (b) Relative expression of LPS synthesis and transport pathway genes. qRT-PCR was performed in the same manner as that described in the Methods under the non-treated condition. The modification genes *lpxK*, *lpxL*, and *lpxM* are important genes involved in acylation during the formation of lipid A; however, their expression level according to PMB resistance has not been confirmed. $PMB^R$, PMB-resistant strains; $PMB^S$, PMB-susceptible strains.

Supplementary Figure 4.




**Supplementary Figure 4. PmrB mutation position on structure schematic.** The domain of PmrB was analyzed using the web-based tool InterPro, and the transmembrane domain and peptide structure were analyzed using the web-based tool Potter. PmrB consists of two transmembrane domains and four functional domains: periplasmic sensor domain, HAMP domain, histidine kinase A, and histidine kinase-like ATPases. All mutations found in our $PMB^R$ strains were located around the sensor domain. The deletion of amino acids at positions 27–29 in NCCP 16007 did not result in conformational changes in the transmembrane domain. In the case of the NCCP 16011 strain, it is presumed that the C-terminal part, which consists histidine kinase-like ATPases, was damaged by mutation and deletion and thus lost its function.

## REFERENCES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