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Role of *Pseudomonas aeruginosa* Low-Molecular-Mass Penicillin-Binding Proteins in AmpC Expression, β-lactam Resistance and Peptidoglycan Structure

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ABSTRACT

This study aimed to characterize the role of Pseudomonas aeruginosa Low-Molecular-Mass penicillin-binding proteins (LMM-PBPs), namely PBP4 (DacB), PBP5 (DacC) and PBP7 (PbpG), in peptidoglycan composition, β-lactam resistance and ampC regulation. For this purpose, we constructed all single and combined mutants of dacB, dacC, pbpG and ampC in from wild-type PAO1 strain. Peptidoglycan composition was determined by HPLC, ampC expression by RT-PCR, PBPs patterns by Bocillin-FL binding test and antimicrobial susceptibility by MIC testing for a panel of β-lactams. Microscopy and growth rate analysis revealed no apparent major morphological changes for any of the mutants compared to wild-type PAO1. Of the single mutants, only dacC led to significantly increased pentapeptide levels, showing that PBP5 is the major DD-carboxypeptidase in P. aeruginosa. Moreover, our results indicate that PBP4 and PBP7 would play a significant role as DD-carboxypeptidase only if PBP5 is absent, together with their inferred DD-endopeptidase activity. As expected, the inactivation of PBP4 lead to a significant increase in ampC expression (around 50-fold), but, remarkably, the sequential inactivation of the three LMM-PBPs produced a much further increase (1000-fold) which correlated with peptidoglycan pentapeptide levels. Finally, the β-lactam susceptibility profiles of the LMM-PBPs mutants correlated well with ampC expression data. However, the inactivation of ampC in these mutants evidenced as well a role of LMM-PBPs, especially PBP5, in intrinsic β-lactam resistance. In summary, in addition to assessing for the first time the effect of P. aeruginosa LMM PBPs in peptidoglycan structure, our results represent a step forward in understanding the impact in β-lactam resistance, apparently driven by the interplay between their role on AmpC induction, β-lactam trapping and DD-carboxypeptidase/β-lactamase activity.

FOOTNOTES

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