Abstract

Drug-resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii* have emerged as important pathogens in recent years. To better understand the genetics of this process in *K. pneumoniae*, mini-Tn5 transposon insertion mutants were constructed in the strain *K. pneumoniae* ATCC13883 and screened for the ability to form biofilm on abiotic surface. Among the mutants which exhibited altered biofilm formation, it was observed that disruption of *wcaJ* conferred high biofilm forming ability as compared to the parent strain. WcaJ is a membrane bound glycosyltransferase and is the initiating enzyme for colanic acid synthesis, and due to its disruption, the synthesis of colanic acid was prevented which resulted in nonmucoid colonies. The disruption of the *wcaJ* gene, resulted in increased resistance towards polymyxin antibiotics and the mutant was less immunogenic in nature compared to the parent. The wild type phenotype was restored upon ectopic complementation. On the other hand, with the growing threat of *A. baumannii*, there is an urgent need to re-evaluate the antimicrobial targets, which is impossible without the knowledge of cell wall physiology. Therefore, to check the intrinsic factors governing the survival of *A. baumannii*, herein we have tried to explore the physiological roles of two putative cell wall remodelling DD-carboxypeptidases, viz. *dacC* and *dacD* in *A. baumannii*. Studies revealed that the deletion of *dacC* resulted in reduced growth rate, loss of the rod-shaped property, reduced biofilm forming ability, enhanced susceptibility towards β-lactams and reduced viability within murine macrophages. The deletion of *dacD* had no such effect. Ectopic expression of the respective genes restored the lost phenotypes. The double knockout mutant in which both *dacC* and *dacD* were absent showed properties similar to the *dacC* single knockout. The *dacC* gene was expressed both in log phase and the stationary phase whereas *dacD* was expressed predominantly in the stationary phase. Both in vivo and in vitro kinetic studies reveal that *dacD* is a stronger DD-CPase as compared to *dacC*. In summary, we conclude that *dacC* encodes a dual enzyme, possessing both DD-CPase and βlactamase activities which significantly affects the physiology of *A. baumannii* in multiple ways, whereas *dacD* encodes for a strong DD-CPase which has negligible impact on bacterial physiology.