Lipopolysaccharide structure and phenotype of \textit{rfaD} knockout \textit{Escherichia coli} mutant

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Lipopolysaccharide is considered as an important virulence factor and permeability barrier of gram-negative enteric bacteria. ADP-L-glycero-L-mannoheptose-6-epimerase, encoded by \textit{rfaD} gene and also called RfaD, is essential for LPS synthesis. In this study, the \textit{rfaD} gene was removed from the genome of \textit{Escherichia coli} W3110. The \textit{ΔrfaD} mutant synthesized a severely truncated LPS, Kdo$_2$-lipid A, judged by SDS-PAGE, TLC and LC-MS-MS. The removal of \textit{rfaD} did not influence the transcription level of related genes \textit{rfaF} and \textit{rfaG} in \textit{rfa} gene cluster, according to fluorescence quantitative PCR analysis. The cell size of \textit{ΔrfaD} mutant was smaller than that of wild type. The growth rate of \textit{ΔrfaD} slightly decreased, but was much faster than WBBO6, another \textit{E. coli} mutant that could synthesize the same Kdo$_2$-lipid A. In addition, compared with the wild type, \textit{ΔrfaD} mutant exhibited higher hydrophobicity, unusually lower permeability, more susceptibility towards novobiocin, stronger ability of autoaggregation, and much weaker biofilm-forming ability. Complementation of the \textit{ΔrfaD} mutant completely restored the above phenotypes. These findings indicate that the \textit{rfaD} mutant can be applied in the production of Kdo$_2$-lipid A and the core of LPS is important for self-defense of gram-negative bacteria.