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Theme and variations in autotransporter adhesins

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Many persistent and chronic bacterial infections are associated with the formation of aggregates and biofilms that are difficult to treat, including respiratory and urinary tract infections (UTIs), infections on medical devices and infections of the ear, gums and heart. Thus, an increased understanding of the mechanisms employed by bacteria to form biofilms is essential for the development of strategies to combat these persistent and intrinsically resistant communities. One mechanism of bacterial aggregation and biofilm formation involves the expression of self-associating surface located autotransporter (AT) proteins. Our work focuses on investigating the structural diversity of AT proteins to understand their mechanism of action. We have recently elucidated the structure of Antigen 43 (Ag43), an AT protein from uropathogenic *E. coli* (UPEC) that self associates forming bacterial aggregates and biofilms. Our studies have shown how Ag43's L-shaped structure drives the formation of cell aggregates via a molecular Velcro-like mechanism. Furthermore, our recent studies on other AT proteins from *E. coli* pathotypes show unexpected structural diversity among this family of proteins, which results in different virulence functions. For example UpaB shares low sequence and structural similarity with Ag43, does not self associate to form bacterial aggregates but binds extracellular matrix proteins (e.g., fibronectin) and increases bladder colonization.

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Design and construction of 1,2,4-butanetriol-producing pathway in *Escherichia coli*

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1,2,4-butanetriol (BT) is an important non-natural chemical with a variety of industrial applications. Here we constructed a prototype strain for BT production from xylose by assembling a four-step synthetic pathway and disrupting the competing pathways in *E. coli.* By fine-tuning of the pathway enzymes expression level, the potential bottlenecks was identified and the BT production was increased by 4.3 fold, achieved a final titer of 1.58 g/L after 72 hours. Furthermore, we designed a novel six-step biosynthetic pathway for BT from malate for the purpose of using glucose as a cheap substrate. Following tests of several combinations of enzymes for the pathway, a five-enzymes-catalyzing-six-step pathway was constructed in *E. coli.* By assembling these enzymes, BT was detected in the fermentation broth upon addition of malate, proving BT can be biosynthesized from malate. As well, BT was detected in the fermentation using glucose as the sole carbon source, suggesting that such novel BT biosynthetic pathway has created the possibility for the production of BT from the cheaper substrate glucose.

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