TraT Virulence Factor Protects Drug-resistant Uropathogenic *Escherichia Coli* Against Serum Killing

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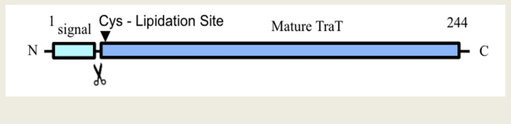
INTRODUCTION

The TraT protein is a multifunctional virulence factor that is encoded by most conjugative F-like plasmids. These plasmids spread antibiotic resistance genes in clinical enteric pathogens, such as uropathogenic *Escherichia coli* (UPEC) and *Klebsiella pneumoniae*, via direct cell-to-cell gene transfer by conjugation. In model *E. coli*, the TraT protein is a highly-expressed outer membrane lipoprotein that is 244 amino acids long.

TraT prevents excessive rounds of plasmid transfer, renders bacteria resistant to a few bacteriophages, and protects against an innate immune response by the host via serum complement killing. The protective effect of TraT within UPEC strains and its mechanism of action are unknown.

Therefore, our objectives in this study were (1) to measure the protective effect of TraT in strains of interest and (2) to establish whether purified soluble TraT is sufficient to provide the full protective effect against serum killing.



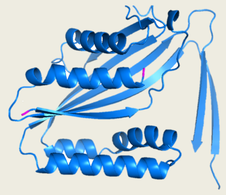


Figure 1. TraT protein primary sequence (left); Predicted structure of TraT-containing PF05818 family. *Source: Baker Group* (right)

METHODS AND MATERIALS

1. Recombinant TraT$^{33-244}$ domain was isolated from BL21(DE3) *E. coli* strain using Ni-affinity chromatography.
2. Exponentially growing *E. coli* cultures with and without traT-encoding plasmids or the purified TraT domain were subjected to different doses of blood serum for 2 hours at 37°C.
3. Viable cells were quantified as colony forming units (CFU) by plating serial dilutions at 0 and 2 hours after addition of serum.

Figure 2. Effectiveness of Ni-affinity purification shown by SDS PAGE gel stained with coomassie blue: protein ladder (circle), whole cell lysate with expressed TraT (triangle), Ni-purified TraT (star)

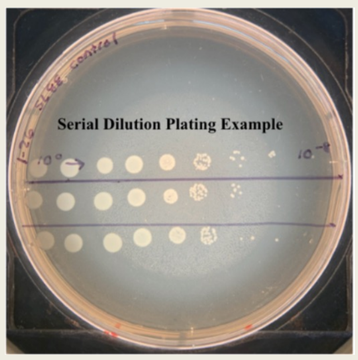


Figure 3. An example plate of how cells were quantified by counting CFUs from serial dilutions

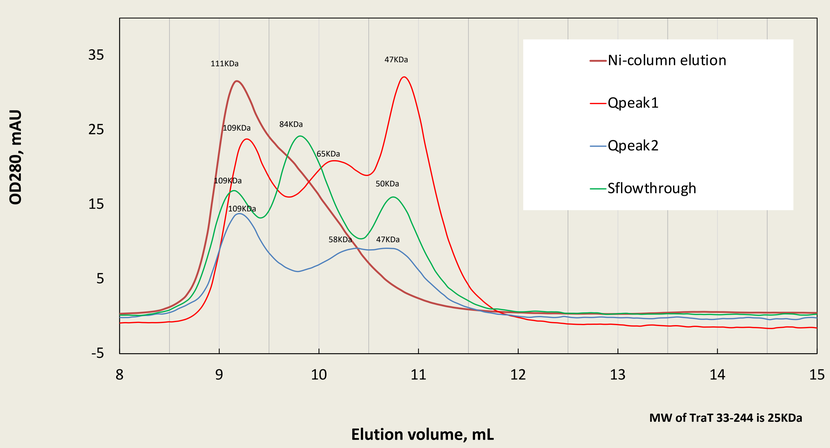
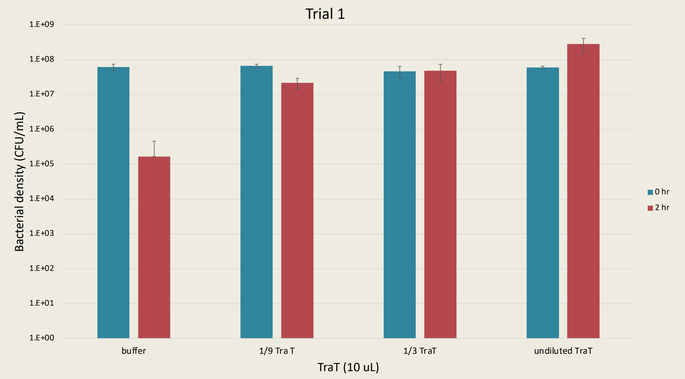
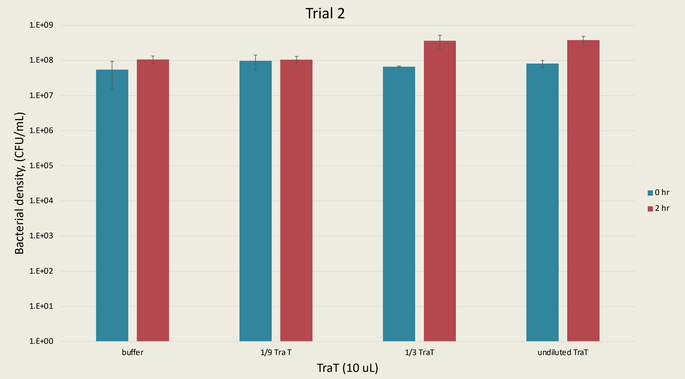


Figure 4. Traces showing TraT after Ni-affinity chromatography and Size Exclusion chromatography using Q column, showing that TraT has 3-5 subunits: the molecular weights of the 3 peaks is roughly 3-5 times that of TraT, suggesting the oligomerization of the protein.

RESULTS

The traT-encoding plasmid provides variable level of protection that depends on serum type, bacterial cells, time, and ratios of bacteria and serum, and can reach several orders of magnitude. We have developed a method of expression and purification of soluble TraT$^{33-244}$ protein suitable for functional and biochemical studies. By testing this purified extracellular domain in serum resistant assays, we found that it is insufficient to exhibit the full protective action of TraT.





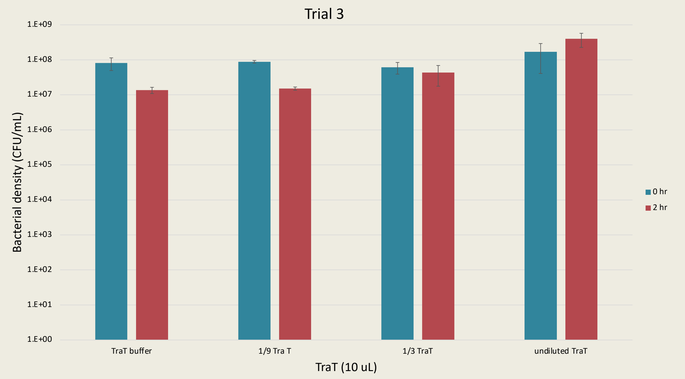


Figure 5. Three different trials of serum killing assays after 2 hours of incubation (0-hour as control). Compared to the buffer (control), the more concentrated TraT used results in a varied but increased level of protection for bacterial cells.

CONCLUSIONS

In this study, we measured the protective effect of traT-encoding plasmid F' in model *E. coli* strains and showed that F-like plasmids provide strong but variable serum protection. The extracellular soluble domain of TraT protein was not strongly protective against serum killing when added to bacterial cells in trans. These results suggest that other parts of TraT protein or TraT interactions within the outer membrane are essential to elicit serum resistance. Overall, this study sets the stage for further details that enable investigating the mechanism of how TraT assists UPEC bacteria in evading innate immune responses within the host organism.

ACKNOWLEDGEMENTS

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