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Periplasmic Expression of Anti-RoxP Antibody Fragments in Escherichia Coli.

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Abstract : Cutibacterium acnes is a commensal bacterium found on human skin that has been linked to acne. C. acnes can also be an opportunistic pathogen when it infiltrates the body during surgery. This pathogen can cause dangerous infections of medical implants, such as shoulder replacements, leading to life-threatening blood infections. Compounding this issue, C. acnes resistance to many antibiotics has become an increasing problem worldwide, creating a need for special forms of treatment. C. acnes expresses the protein RoxP, and it requires this protein to colonize human skin. Though this protein is required for C. acnes skin colonization, its function is not yet understood. Inhibition of RoxP function might be an effective treatment for C. acnes infections. To develop such reagents, the McCoy Laboratory generated four unique anti-RoxP antibodies. Preliminary studies in the McCoy Lab have established that each antibody binds a distinct site on RoxP. To assess the potential of these antibodies as therapeutics, it is necessary to specifically characterize these antibody epitopes and evaluate them in assays that assess their ability to inhibit RoxP-dependent C. acnes growth. To provide material for these studies, an antibody expression construct, Fv-clasp(v2), was adapted to encode anti-RoxP antibody sequences. The author hypothesizes that this expression strategy can produce sufficient amounts of >95% pure antibody fragments for further characterization of these antibodies. Four anti-RoxP Fv-clasp(v2) expression constructs (pET vector-based) were transformed into E. coli BL21-Gold(DE3) cells and a small-scale expression and purification trial was performed for each construct to evaluate anti-RoxP Fv-clasp(v2) yield and purity. Successful expression and purification of these antibody constructs will allow for their use in structural studies, such as protein crystallography and cryogenic electron microscopy. Such studies would help to define the antibody binding sites on RoxP, which could then be leveraged in the development of certain methods to treat C. acnes infection through RoxP inhibition.

Keywords : structural biology, protein expression, infectious disease, antibody, therapeutics, E. coli
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