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ENHANCING PRODUCTION OF THE MALARIA ASEXUAL BLOOD-STAGE VACCINE CANDIDATE PFRIPR5 IN INSECT CELLS BY MODULATING EXPRESSION VECTOR AND CULTURE TEMPERATURE

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Despite the recent approval of the first malaria vaccine RTS,S/AS01, its efficacy in children and infants is still modest. Therefore, continued development of new, improved malaria vaccines, including asexual blood-stage vaccines such as the one herein targeted, is essential to reach desired levels of protection against disease and mortality.

In this study, the insect cell-baculovirus expression vector system (IC-BEVS) was used to produce a malaria asexual blood-stage vaccine candidate based on PfRipr5 antigen and compared to traditional mammalian (HEK293) cell system. PfRipr5 could be expressed to higher levels in IC-BEVS, with higher protein purity and reactivity to a conformational anti-PfRipr monoclonal antibody than its mammalian counterpart. The performance of IC-BEVS was further improved by modulating the expression vector sequence and culture temperature. The addition to the expression vector of (i) one alanine (A) amino acid residue adjacent to the signal peptide cleavage site, and (ii) a glycine-serine linker (GGSGG) between the PfRipr5 sequence and the purification tag, resulted in up to 2.2-fold increase in the expression of secreted PfRipr5. In addition, lowering temperature from standard 27 °C to 22 °C at the time of infection improved PfRipr5 productivity by up to 1.7-fold. Noteworthy, a synergistic effect was attained by combining both optimization strategies, enabling to increase expression of extracellular PfRipr5 by up to 4-fold and process yield post-purification by 5.2-fold, while maintaining same degree of protein purity and reactivity.

This work highlights the potential of insect cells to produce the PfRipr5 malaria vaccine candidate and the importance of optimizing the expression vector and culture conditions to boost expression of secreted proteins.