# Abstract

**Title: Gram positive cell factories: production of disulfide-rich recombinant *Plasmodium falciparum* malaria vaccine candidates in *Lactococcus lactis***

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## I. Abstract Body (up to 250 words)

The production of recombinant proteins with proper conformation, appropriate post-translational modifications in an easily scalable and cost-effective system is challenging. *Lactococcus lactis* has recently been identified as an efficient Gram positive cell factory for the production of recombinant protein. We and others have used this expression host for the production of selected malaria vaccine candidates. The safety of this production system has been confirmed in multiple clinical trials. Here we have explored *L. lactis* cell factories for the production of around 50 representative *Plasmodium falciparum* antigens from different stages (Pre-erythrocyte, asexual and sexual) with varying sizes (ranging from 9-90 kDa) and varying degree of predicted structural complexities including thirty antigens with multiple predicted structural disulfide bonds, those which are considered difficult-to-produce proteins. Of the 50 recombinant constructs attempted in the *L. lactis* expression system, the initial expression efficiency was 60% with 30 out of 50 recombinant gene constructs producing high levels of secreted recombinant protein. The majority of the constructs which failed to produce a recombinant protein were found to consist of multiple intra-molecular disulfide-bonds. We found that these disulfide-rich constructs could be produced in high yields when genetically fused to an intrinsically disorder protein domain (GLURP-R0). By exploiting the distinct biophysical and structural properties of the intrinsically disordered protein region we developed a simple heat-based strategy for fast purification of the disulfide-rich protein domains in yields ranging from 1 to 40 mg /L.

**Conclusions:** A novel procedure for the production and purification of disulfide-rich recombinant proteins in *L. lactis* is described.

## II. Reference:

1 Singh SK, Roeffen W, Andersen G, Bousema T, Christiansen M, Sauerwein R, Theisen M: A *Plasmodium falciparum* 48/45 single epitope R0.6C subunit protein elicits high levels of transmission blocking antibodies. *Vaccine* 2015, 33:1981-1986.

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3 Theisen M, Adu B, Mordmuller B, Singh S: The GMZ2 malaria vaccine: from concept to efficacy in humans. *Expert Rev Vaccines* 2017, 16:907-917.

## III. Biography:

Dr. Susheel Kumar Singh has completed his PhD at the age of 26 years from Jabalpur University, India. He did his post-doctoral training with an emerging Indian biotech industry and a premier malaria research center from Denmark. Currently, He is an Assistant professor at Department of International Health, Immunology and Microbiology, University of Copenhagen, Denmark. He has published more than 20 papers in reputed journals and has been serving as a reviewer and an editorial board member of reputed journals.

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