

Identification and development of a subunit vaccine against *Plasmodium vivax* through immunogenomics and heterologous expression in transgenic plants

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According to the World Health Organization, there were about 13.8 million cases of malaria caused by *Plasmodium vivax* worldwide in 2015, but no vaccine against this parasite is available. The empirical method for identifying relevant antigens for a subunit vaccine is time consuming and expensive. Therefore, *in silico* strategies involving immunogenomics tools have become crucial to reducing both time and costs associated with vaccine development. This work aims to select peptides from different stages of development of *P. vivax* through bioinformatics, and to express these peptides in plants as a subunit chimeric vaccine. In order to assemble a library of vaccine candidates, we used the PlasmoDB genome database to select *P. vivax* proteins i) with predicted signal peptide, ii) predicted to contain a PEXEL or HT motif, iii) predicted to be secreted, iv) whose corresponding genes had telomeric or subtelomeric location and/or v) containing predicted epitopes. Grounded on the above criteria, we constructed a library of vaccine candidates containing 486 antigens. Based on further detailed *in silico* analyses of each of these peptides, such as epitope prediction for T CD4⁺ lymphocytes (MHC class II), T CD8⁺ lymphocytes (MHC class I) and B lymphocytes, we selected a blood-stage protein (MAEBL) and two liver-stage proteins to compose the chimeric vaccine. We then constructed the fusion gene, separating each of the three peptide-coding genes by a rigid linker, and used the optimized the codon sequence of *P. Vivax* according to the codon usage of *Arabidopsis thaliana*. The fusion gene was cloned into the pC3300 under the control of the constitutive 35SCaMV promoter. This vector, named as pMALARIA, was inserted into *A. tumefaciens* EHA105 strain and used to transform lettuce and tobacco. The immunogenicity, antigenicity and protection of the resulting fusion peptide are being carried out.

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