THE RECOMBINANT BACTERIA *LACTOCOCCUS LACTIS* EXPRESSING SARS-COV-2 SPIKE PROTEIN OR ITS RECEPTOR-BINDING DOMAIN ELICITS AN IMMUNE RESPONSE FOLLOWING ORAL IMMUNISATION OF RATS

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Mucosal delivery of antigens represents an effective strategy for the development of vaccines to control diseases with the upper respiratory and/or gastrointestinal tracts as the main routes of transmission. Lactic acid bacteria (LAB) are food-grade bacteria used for the expression of target antigens in the development of mucosal vaccines. Over the last two decades, research on the use of LAB has focused on the construction of genetically modified strains. Current study aimed to develop and examine the recombinant *Lactococcus lactis* strains, expressing SARS-CoV-2 spike (S) protein or the receptor-binding domain (RBD) of S protein as immunizing antigens.

The spike gene from the component II of Sputnik V vaccine and two synthesized coding sequences of the RBD region of SARS-CoV-2 spike protein (319–529 residues named HA-spike and 318–590 residues named mini-spike) were subcloned into expression vector pNZ8121. The resulting plasmids were verified by sequencing and subsequently transformed into competent *L. lactis* NZ3900 cells. The expression of recombinant proteins in obtained strains was analyzed by western blot using SARS-COV-2 Spike RBD antibodies. The results showed that the spike gene could be efficiently expressed in the cells of recombinant *L. lactis*. The major 150 kDa band detected in the cell lysate of *L. lactis* pNZ::spike represented the full-length S protein. Protein bands of 35 kDa and 23 kDa from the lysates of *L. lactis* pNZ::mini-spike and *L. lactis* pNZ::HA-spike respectively were detected indicating that the recombinant RBD protein was also successfully expressed. The mini-spike was expressed more abundantly than the HA-spike variant. Based on the growth kinetics and protein expression profiles of recombinant strains, the highest protein yield was obtained under the following conditions: overnight culture was inoculated (1/25 inoculum) and grown until reaching an optical density of 0,9 at 600 nm, then gene expression was induced by adding nisin (1 ng/ml), cells were harvested after 3 h incubation with the inducer.

To examine the immunogenicity of recombinant strains, 1 ml of cell suspension ($1,5\times10^9$ CFU) of *L. lactis* pNZ::spike, pNZ::mini-spike or *L. lactis* pNZ::HA-spike was orally administered to Wistar rats. The same volume of peptone water was administered to the control group. Serum was collected from the animals at 28 days and IgG level was determined by ELISA for the quantitative determination of IgG antibodies to SARS-CoV-2 (COVID-19) Spike RBD protein in rat serum. As expected, the serum from control animals lacked antibodies to the RBD antigen. The serum of rats immunised once with cell suspension of the recombinant strains showed the following levels of specific IgG: 124,24±12,56 ng/ml in group pNZ::mini-spike, 165,91±59,13 ng/ml in group pNZ::spike, and 213,60±54,08 ng/ml in group pNZ::HA-spike. The highest IgG level (545,56±230,24 ng/ml) was detected in the serum of rats immunized with ultrasound-treated cell suspension of *L. lactis* pNZ::spike. No adverse effects were observed in vaccinated animals at regular check-ups throughout the study period.

The current results have demonstrated that the RBD and full spike protein were successfully expressed in *L. lactis*. Oral administration of the recombinant *L. lactis* expressing S protein or RBD to rats appeared to generate the anti-SARS-CoV-2 antibodies in serum, positioning this bacteria as promising basis for creating oral vaccine.