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# Immunogenicity of recombinant bacterial antigens expressed as fusion proteins in transgenic rice seeds

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**Abstract**

Rice-based vaccines do not require high-cost purification. They are stable at room temperature, can eliminate the risk of attenuated vaccine strains, and are resistant to gastrointestinal degradation. We tested the applicability of an oral delivery system for tuberculosis (TB) and cholera antigens in transgenic rice for induction of immune responses in the mucosal compartment as well as in the systemic circulation. For vaccine development, we selected mycobacterial Ag85B antigen and immunoprotective P4 epitope of TcpA fused to the nontoxic cholera toxin B (CTB) subunit for immunization against TB and cholera, respectively, in independent constructs. The expression levels of CTB, CTB-TcpA, and CTB-Ag85B in transgenic lines containing stably integrated, chimeric genes showed up to 0.64%, 0.34%, and 0.02% of total rice seed protein, respectively. Oral immunization of mice with each of the three seed lines resulted in significantly increased levels of both anti-CTB IgG and IgA responses in the serum and IgA responses in the bronchoalveolar lavage (BAL) fluid. This indicated the capacity for oral immunization to elicit immune responses in the respiratory mucosal compartment. Plant-expressed TcpA could be detected in immunoblot analysis by using TcpA-specific commercial antibody, while there was no recognition of rice-expressed Ag85B by the commercial antibody raised against the latter antigen, where both antibodies were produced against the antigens expressed in the bacterial system. This study focused on identifying antigens resistant to both posttranslational modifications in plants and immunogenic under the proposed delivery system in animals for boosting the mucosal and systemic humoral immune response against enteric as well as respiratory pathogens.

**Key words**: Ag85B, cholera, oral vaccines, TcpA, transgenic rice, tuberculosis

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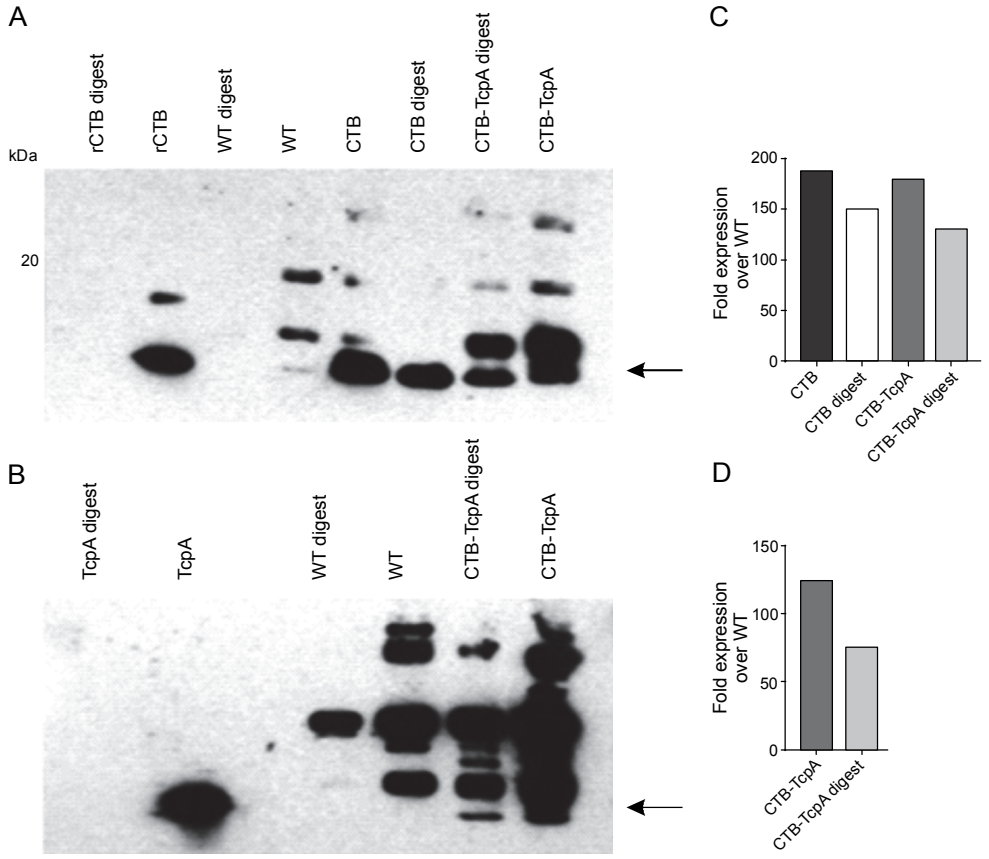


**Fig. S1.** Pepsin digestion of transgenic CTB (12.9 kDa) and CTB-TcpA-P4 (15.1 kDa) seed powder; western blots of the digestion reactions were performed using A) rabbit anti-CTB and B) rabbit anti-TcpA antibodies, with purified rCTB and TcpA proteins serving as the respective non-rice controls; red arrows denote positions of the protein bands of interest; densitometric analyzes confirm the retention of the CTB C) and TcpA D) antigens within the rice seed even in a proteolytic environment


**Fig. S2.** Protein identification of CTB-Ag85B by mass spectrometry; the blue segment corresponds to CTB, downstream to the *GluB-1* signal peptide, and is linked through a glycine hinge to Ag85B; the latter is highlighted in green; red amino acids indicate the matched peptides

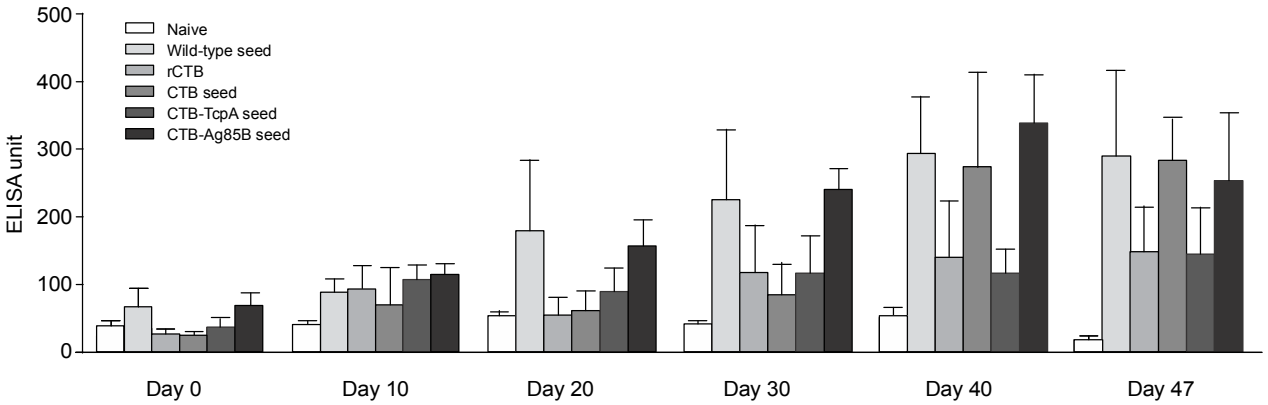


**Fig. S3.** CTB-specific antibody responses in the sera of immunized mice; ELISA for the immunoglobulin IgM was performed for each cohort using the serum from an individual mouse collected at six different time points; the columns indicate mean responses, and the error bars represent standard errors of the mean

MTDVSRKIRAWGRRLMIGTAAAVVLPGLVGLAGGAATAGAFSRPGLPVEYLQVP
SPSMGRDIKVQFQSGGNNSPAVYLLDGLRAQDDYNGWDINTPAFEWYYQSGLSI
VMPVGGQSSFYSDWYSPACGKAGCQTYKWETFLTSELPQWLSANRAVKPTGSA
AIGLSMAGSSAMILAAYHPQQFIYAGSLSALLDPSQGMGPSLIGLAMGDAGGYKAA
DMWGPSSDPAWERNDPTQQIPKLVANNTRLWVYCGNGTPNELGGANIPAEFLE
NFVRSSNLKFQDAYNAAGGHNAVFNFPPNGTHSWEYWGAQLNAMKGDLQSSL
GAG

**Fig. S4.** Potential glycosylation sites in Ag85B; potential glycosylation sites in Ag85B are shown in underlined letters