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CREATION OF A SYSTEM FOR PRODUCING THE RECOMBINANT β -SUBUNIT OF CHOLERA TOXIN IN *E. COLI* CELLS

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Abstract: Cholera is a deadly infectious disease, which is usually associated with low hygiene levels and limited access to high-quality drinking water. An effective way to prevent cholera is the use of vaccines. Among active vaccine components there is the CtxB protein (cholera toxin β -subunit). In the current work, we have developed a genetic system for production of the recombinant CtxB in *E. coli* cells and studied conditions for synthesis and purification of the target product at the laboratory scale. It has been found that the optimal algorithm for isolation of the recombinant protein is to grow *E. coli* culture in the synthetic M9 medium with glycerol, followed by CtxB purification out of the spent culture medium using Ni²⁺—chelate affinity chromatography techniques. Forty-eight hours after induction of CtxB expression, concentration of the target product could be up to 50 mg/liter in the culture medium. The CtxB protein retains its pentameric structure during expression and through purification. The latter makes it possible to consider the developed system as a promising tool for the industrial-level production of recombinant CtxB for medical and research purposes.

Keywords: β -subunit of cholera toxin, CtxB, expression, recombinant protein.

Introduction: Cholera is an acute infectious diarrheal disease that poses a particular danger to humans. It is caused by the bacteria *Vibrio cholerae*. Without adequate treatment, the disease can quickly lead to dehydration, seizures and death. The main ways to prevent cholera are to provide the population with clean water and uninfected food, as well as vaccination. The most promising is the use of a vaccine based on a genetically modified non-toxigenic strain of *V. cholerae* supplemented with the recombinant CtxB protein. The non-toxicity of CtxB and its ability to trigger the development of a pronounced immune response allows this protein to be widely used as an adjuvant and basis in the development of vaccines against various bacterial and viral pathogens.

Purpose of the work: to create genetic constructs for the production of the β -subunit of cholera toxin, to determine the optimal conditions for its induction, its localization in the cell and the preferred method of its isolation from bacterial cultures.

Materials and methods: In order to produce the target protein, the *E. coli* strain BL21(DE3), transformed with the pET22b(+) vector, was used. The construction of expression plasmids was carried out using amplification, restriction, ligation and transformation. Isolation of the target CtxB protein was carried out by metal chelate chromatography using Ni-NTA agarose as a carrier.

Results: The protein produced in the expression system we created was identified by the MALDI-TOF method. Western blotting of column-purified CtxB showed that the protein effectively interacts with specific monoclonal antibodies, which confirms its antigenic correspondence to the β -subunit of wild-type cholera toxin. The efficiency of CtxB production using various plasmid variants was analyzed, and the most promising ompA clone was selected. It has been experimentally established that the yield of the target protein increases when *E. coli*

cells are cultivated under conditions of reduced temperature 20-25°C. The greatest accumulation of recombinant CtxB was observed in the lysate of cells grown on a rich nutrient medium. The maximum content of CtxB in the supernatant of producer cultures was also observed on 21NB medium. With prolonged growth on M9 medium, the content of the target protein in the cultivation medium constantly increases and 48 hours after induction reaches 50 mg/liter. At the same time, the protein is stable and retains its natural pentameric structure.

Conclusion: The result of the work was the creation of a genetic system and a method for producing recombinant CtxB. It has been established that growing a culture of the producer strain on a synthetic nutrient medium M9, followed by purification of the protein from the culture liquid using metal chelate chromatography, appears to be optimal for one-step isolation of the highly purified β -subunit of cholera toxin.

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EVALUATING THE PERFORMANCE OF WHEAT DRILLS BY SEED DISTRIBUTION METRICS: CASE OF TSELOT FARM, ERITREA

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Abstract: *This study evaluated the uniformity of plant populations in two plots by randomly positioning a 1-m² wire at 10 locations within each plot. Statistical analysis revealed that plot-3 had a higher overall plant population as compared with plot-2 and exhibited greater variability, indicating possible inconsistencies in planting practices. The automation or precision-guided operations is expected to improve the consistency of planting across the plots.*

Keywords: *Agricultural mechanization, plant density, Eritrea, t-tests, Mann-Whitney U-tests.*

Introduction

Agricultural mechanization plays a crucial role in enhancing agricultural productivity and income generation, especially in economies heavily reliant on agriculture [1]. It involves using a range of tools and machinery to reduce labor, increase efficiency, and improve the timeliness of such operation as planting and harvesting. By reducing peak labor demand [2] and ensuring timely operations, mechanization boosts yields and profitability. However, effective evaluation of farm