



SLOVENSKÁ AKADEMIA VIED

Ústav molekulárnej biológie

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**Evaluation of the Habilitation thesis of Mgr. Zdenko Levarski, PhD.:
„Mikrobiálna produkcia rekombinantných proteínov v ére
syntetickej biológie a „precision“ prístupov”**

The thesis comprises 141 pages, and its structure is primarily composed of several scientific articles (10) in which the candidate is a co-author. To prepare the reader for the central theme of the thesis, the first part of the study, approximately 25 pages in length, explains the principal aims of the habilitation work using literature, figures, and examples. This part is divided in 5 sections: Úvod; Ciele práce; Prehľad literatúry (Produkcia rekombinantných proteínov, N-glykozylácia proteínov v *E. coli*, *Vibrio natriegens*, Extracelulárna produkcia proteínov); Záver; Zoznam použitej literatúry. In this thesis, the 10 papers co-authored by the candidate demonstrate his valuable skills in recombinant DNA methods and biotechnological applications, acquired over the past years.

The primary objective of this study was to investigate the potential of recombinant protein production, with a focus on bacterial host strains. The long-term goal has been the production of proteins that are difficult to express. The thesis describes the use, mainly of *E. coli*, as a proteo-synthetic apparatus for the production of recombinant yeast and bacterial alcohol dehydrogenase, yeast formate dehydrogenase, and human growth hormone. It was also optimized to produce N-glycosylated bacterial alcohol dehydrogenase. Several protein expression, production, and purification problems were overcome by optimizing culture conditions (reducing culture temperature, limiting carbon source, reducing inducer concentration, and adding appropriate supplements), vector design, fusion with a suitable expression partner, manipulation of the host genome, or targeted mutagenesis.

The second part of the study focused on the design and construction of a completely new expression system, based on the *Vibrio natriegens* strain. In this case, the goal was to introduce this strain into laboratory practice through a series of optimizations

of basic methods (transformation, selection of recombinants) to create a strain capable of passively releasing the produced recombinant proteins into the culture medium.

I have a few questions for the candidate:

- You have spoken in the thesis about the use of AI to help scientists during the design of various DNA recombinant tools. Unfortunately, I did not find any specific example. Please describe, in brief, how AI can assist scientists in this field. Or at least in which crucial steps can AI help?

- At the moment, the glycosylation, in your hand, is a property acquired by your laboratory *E. coli*. Is this ability also possible to transfer to *Vibrio natriegens*?

- Are you working, at the moment, to some interesting DNA recombinant process in which *Vibrio natriegens* is involved? If yes, may you show us?

The study is fascinating and provides new recombinant DNA molecular tools that can advance several biotechnological applications. Therefore, this study has extensive knowledge and practical usefulness.

This scientific study meets the requirements for a habilitation thesis; thus, I recommend it for defense. After the successful defense, I recommend that it be submitted to Mgr. Zdenko Levarski, PhD., the academic title "Docent" (Doc.).

Bratislava 29.12.2025

Dr. Domenico Pangallo, DrSc.