Ph.D. Thesis: **Ecophysiological characteristics of key members of *Betaproteobacteria* in freshwater bacterioplankton**

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Brief evaluation of the supervisor: prof. RNDr. Karel Šimek, CSc.
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This thesis deals primarily with one of the key groups of *Betaproteobacteria*, the *Limnohabitans* genus, namely its R-BT065 cluster, which was found to be highly active in plankton of most of pH-circum neutral freshwater systems. Notably, before elaborating this thesis all available knowledge about this group was entirely based only on the use of the R-BT065 FISH-probe targeting this cluster in environmental samples. While the probe was designed already in 2001, we have been completely lacking a successful isolation of the relevant members of the group that would allow for testing of their ecophysiological traits and microdiversity within the genus. During the PhD studies, Vojta Kasalický succeeded in isolating of more than 40 representative strains of the *Limnohabitans* bacteria from various habitats (from the so far uncultured R-BT lineage).

Until today, I can only speculate about the secret how Vojta "convinced" all these sensitive microbes to stay with him in a form of a pure culture – likely he talk to them so nicely that they simply can not resist this urgent call for getting rid of all nasty contaminating microbial bugs. It's really notable, since all others before Vojta basically failed to get the real pure cultures of these bacteria that are well performing on natural algal derived substrates, such e.g. algal exudates. The successful isolation of these bacteria also attracted many collaborators from abroad and yielded nice studies with international co-authorship.

This progress stimulated new research dealing, for instance, with microdiversity within the lineage based on proposition of new phylogenetic markers to unveil so far hidden diversity, further it allowed studies of niche separation and substrate preferences of closely related co-occurring species and new probe design for RLBH method applicable for environmental samples. Thus, the results presented in the thesis go far beyond the taxonomic descriptions only, and one can see several quite new and really important directions of research of the group that were significantly accelerated by making these strains available for further research, such as sequencing of the strains, probe design and various manipulative in situ or laboratory experiments.

The thesis consists of Background rationale, study Hypotheses and Objectives, well written Results and Discussion, plus 6 published papers and one paper, submitted to a core journal of Environmental Microbiology (IF=5.537) that is in revision, at least in my eyes, with generally positive reviews. Altogether, Vojta is the first author of two papers and co-author of 5 more papers. Regarding the quality of Vojta's work during the PhD study - it is definitely worth mentioning that Vojta got the first prize for the best poster on the Symposium of Aquatic Microbial Ecology, Rostock, Germany. The poster presented a new phylogenetic system of the genus *Limnohabitans,* based on 35 sequenced strains with the novel markers proposed, based o ITS area of ribosomal RNA, which underlies his thoughtful approach and excellent way of the results presentation. Last but not least, Vojta is a very popular person in our working group with a rather unconventional way of thinking, which is quite stimulating and refreshing and brings lots of fun.

Vojta also invested lots of time into introducing and leading new young scientists entering the field of aquatic microbial ecology, namely numerous master and bachelor students, which was of great help to me and to other members of the team. **Just one note** – Vojta should in general speed up with publishing of his nice results since there is still plenty of data left that are worth publishing in core journals in the field. This is a "long run task" and a part of scientific life that one is learning step by step.

Since I am a co-author on all papers presented in the thesis, please do not expect me to criticize their contents – I will leave this possibility to our carefully selected opponents that are specialist in many aspects of microbial ecology and modern molecular approaches.

The submitted PhD thesis meets all the prerequisites for defense requested by the South Bohemian University. At least in my opinion, it is a fairly nice peace of work that is fulfilling all aspects for successful PhD thesis defense aside of creating an excellent base for Vojta's future scientific carrier. I believe that also reviewers will have similar views on the submitted PhD thesis.

Prof. RNDr. Karel Šimek, CSc.
Supervisor/ Školitel

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