

Review of the thesis „Ecophysiological characteristics of key members of *Betaproteobacteria* in freshwater bacterioplankton“ by Vojtěch Kasalický

The thesis summarizes an extensive isolation effort oriented towards the highly important freshwater bacterial taxa – the members of the genus *Limnohabitans* including the characterization of individual strains and exploration of their ecophysiological traits. The results obtained by the author contributed to six publications in IF journals, out of these, the author of the thesis was first author in one case. One manuscript where the author of the thesis is the first author is currently in revision in the IF journal (Environmental Microbiology – IF 5.537). The contribution of the author to the individual publications is clearly stated in the thesis. While the author was the main contributor to the isolation of bacteria, he also significantly contributed to the description of bacterial taxa and the ecophysiological studies that were performed by a larger group of cooperators. The thesis brings important new results since so far the isolation of bacteria belonging to the above group was largely unsuccessful. Furthermore, the last paper of the thesis (in revision after receiving positive reviews) clearly shows the quantitative importance of the studied group of bacteria in various limnic ecosystems and in my opinion represents an enormously important contribution to the knowledge of limnic *Betaproteobacteria* which will impede future research. The isolation success also allowed the candidate and his colleagues to explore in depth the functional traits of the taxa including these important for their ecological roles. I do not have major concerns regarding experimental methodology - many parts of it are highly innovative. Although the formal appearance of the work is quite far from perfection, the thesis still represents a good standard of what can be presently found in this type of literature and the scientific results itself are highly valuable. Along with the fact that parts of the thesis have already been published in scientific papers, it is definitely satisfactory in terms of granting the author the doctoral degree. Specific comments, both general and particular, are listed below.

Specific comments:

- 1) The title of the thesis is much too general. I suppose that there are several other taxa of *Betaproteobacteria* that can be counted among key members, e.g. the *Polynucleobacter*.
- 2) The annotation speaks eloquently about the scientific value of the work. Instead, one would expect a summary of what was done and what is novel to science.
- 3) The language is comprehensible, but the quality is not at all perfect. At least the spell check should have been thoroughly performed.
- 4) P3 L14-17: The exact meaning of the sentence „However ...“ is unclear to me. Has the extent of rDNA and genome variability already been assessed in order to make such conclusion (i.e., has a relevant amount of genomes been sequenced and annotated) or does the author mean that the rDNA conservation is limited compared to **phenotypic** divergence of taxa?
- 5) P4 L1-2: Although I agree with the importance of the research on isolated strains, much of the information derived from such studies rather define the potential niche than the realised one (in the native environment, in competition etc.). What does the author think about the relative importance of single-species studies or lab microcosms compared to e.g. the use of in situ transcriptomics?
- 6) P4 L9-10: Please explain what you mean with „patchiness of surrounding aquatic environment“.



- 7) P5 „Defence specialization“: it would be helpful to include some examples of taxa with known strategies. Are the strategies only proposed or experimentally demonstrated?
- 8) P6 L20: „*Methylophilus*“
- 9) P6 L25: The meaning of the sentence „As a result...“ is unclear. Does the author anticipate that there are family-specific traits in bacteria?
- 10) P6 L27: What is the meaning of „extras and loss“.
- 11) P7 L11: „1 Gyr ago“; the abbreviation Gyr is not common, it should better be explained.
- 12) P7 L13: „inventions“
- 13) P7 L26-27: „This fact ...“. This idea might be worth more comments. First of all, how is the genus in bacteria typically defined? Does it correspond to some objective definition (e.g., % of genome similarity, phenological similarity, combination of the above...). Is the genus level „definition“ in bacteria the same across all higher bacterial taxa (e.g. phyla)? Should one expect that it will be homogeneous physiologically or ecologically?
- 14) Fig. 1 caption L4 „amount“ instead of „mount“
- 15) P8 L7: „tropical“
- 16) P9 last paragraph: The paragraph is unclear since it contains many previously unexplained abbreviations, e.g. the specificities of individual probes. Is the betaI cluster the same as betI in Fig. 1?
- 17) P10 par. 1: The paragraph needs better organisation. The individual points are now not enough connected and the story is thus uneasy to follow.
- 18) P10 L2: „long time“
- 19) P10 L3-4: what kind of „specific activities“ are concerned here?
- 20) P10 L10-11: unclear what is meant by „in such environment“
- 21) P10 L19: „research of investigations“ is a nonsense
- 22) P11 L10-12: „Regarding...“ does this conclusion cover all *Limnohabitans* bacteria or just the RBT?
- 23) P11 par. 3: There is a logical gap between par. 2 and 3. How does the content of par. 3 follow from the previous text („Thus, ...“)?
- 24) P12 L5: „FAM“ not explained
- 25) P12 L23: What is the definition of „microdiversity“?
- 26) P12 L24: Can anything on functional level be correlated to the 16S rRNA sequence?
- 27) P13 L6: „Our collection“ – please give the real contribution of the author to the collection.
- 28) P13 L7: I suppose that rather the strains itself then the data on them are deposited in the culture collection. Please clarify.
- 29) P13 last sentence: does it apply to the group „LimD“ in Fig. 2?
- 30) Fig. 2: It would be advisable to separate the ecological characteristics of the habitats from the localizations, now it is mixed.
- 31) P14 L6-7: What is the definition of the „lineage“ and the „genotype“? What makes IGS such a good marker compared to the SSU?
- 32) P15 L12: „HNF“ not explained – should be heterotrophic nanoflagellates (HNF),
- 33) P15 L27: „easily determinable value“ or „obvious determinant of“?
- 34) P16 L17-18 rather „association with certain organisms“ than „addiction on different organisms“
- 35) P16 L21: The difference between „bio-habitats“ and „interactive-habitats“ is unclear or does both mean the same?
- 36) P17 L2 from the bottom: „tribes“ or „genera“?

- 37) P18 L17 rather „limited set of compounds“ than „few group elements“
- 38) P18 L2 from the bottom: „abundance“ is better than „contribution“
- 39) P20 L10: „Concentration of none of the main ...“
- 40) P20 L12: How does the niche separation look like under natural conditions? Do specific taxa occur at various times (e.g. with high/low autochthonous C input)?
- 41) The thesis does not mention results of metagenomics / metatranscriptomics studies in the freshwater environment. Can the candidate mention what results (if any) have been presently obtained using these methodologies and what is their expected importance for this particular field of study, compared, e.g. to the culture- or microscopy-based approaches?

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Evaluation of the PhD thesis of Vojtěch Kasalický

**Dear PhD Committee,**

**Prof. Dr. Hans-Peter Grossart**

It was a pleasure to read and evaluate the PhD thesis of Vojtěch Kasalický. The thesis is an exciting example for what can be done in a very well planned and performed PhD thesis. Vojtěch Kasalický has included 7 manuscripts, all published or submitted to international peer-reviewed journals. On two of the manuscripts he is the first author and on five he is a coauthor. The thesis is very well written, starting with a general introduction into the topic leading to the hypotheses and aims of the study. The results are very well presented and discussed which allows Vojtěch Kasalický to transfer his own findings and to discuss them in a broader range, which also enabled him to raise some important questions and to give a variety of future perspectives in the field of freshwater microbial ecology. Taking all his studies together it becomes obvious that he belongs to the upper 5-10% of the PhD students who are well suited for a future successful scientific career.

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Myself, I have met Vojtěch Kasalický for the first time in Třeboň for a workshop on AAPs which had been organized by Michal Koblížek and his group. During this time he just had started his PhD thesis. Today, I am impressed who fast he has been managed to generate this incredible amount of interesting data on one of the most interesting bacterial clades in freshwater: the Betaproteobacteria. More specifically, Vojtěch has focused on on the *Limnohabitans* and *Polynucleobacter* genera, which both perform different life-styles and have adapted differently to a variety of freshwater environments. Interestingly the *Limnohabitans* genus has been found al-

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most worldwide and in very different habitats ranging from oligotrophic to hypertrophic lakes, from arctic to tropical ecosystems, and from high mountains to lower land lakes and even rivers. Interestingly, *Limnohabitans* had been also found as an endosymbiotic bacterium in Hydra and even daphnids, implying a parallel development to *Polynucleobacter*. When Vojtěch started his work, the phylogeny of *Burkholderiaceae* and *Comamonadaceae* could not be supported by any physiological trait. Therefore, for his thesis he has addressed 4 major hypotheses, which addressed the culturability of *Limnohabitans*, its spectrum of substrates, the effect of protistan grazing, in situ occurrence and abundance, as well its morphology and physiological capacity by using selected isolates. Vojtěch has been extremely successful in isolation and has obtained a sheer number of various strains from a multitude of environments. The isolates formed an excellent basis for his past PhD thesis, but also for potential future research related to this exciting group of freshwater bacteria.

Vojtěch's main contribution to the field of microbial ecology is the isolation of bacterial strains from the *Limnohabitans* genus, mainly from its RBT lineage and a very precise characterization of their ecophysiological capabilities. His collection contains more than 40 viable strains from all kinds of environments. His work has allowed the species description of *L. australis*, *L. curvus*, *L. parvus* and *L. planktonicus*, which are now available from public collections such as the DSMZ and the Institute Pasteur. This is extremely helpful for further studies by the scientific community.

For his work, he also had to modify the "Filtration and acclimation method" (Hahn et al. 2004a), which proved to be a valuable tool for isolation of *Limnohabitans* strains. The modification is based on separation of bacteria from grazers by using a 0.8 µm pore-size filter, followed by the overnight activation in the water from their home environment and the subsequent dilution to extinction ("Separation, activation, dilution and acclimation method" - SADAM). Further, he has successfully tested the intergenic spacer region between 16S and 23S rRNA genes (IGS) for its use as a fine-scale marker to delineate individual lineages and even the genotypes. This method was largely needed since the low genetic diversity of previous studies (Zwart et al. 2002, Newton et al. 2011) based on the use of the ribosomal SSU gene, is contrasted by a great variety in morphotypes and different patterns in substrate utilization of the isolated strains.



Last, he has developed a new experimental design for performing predator prey competition experiments even in the presence of viruses, hence, covering for a large portion of the microbial food web.

The presented PhD thesis nicely shows that the distribution of *Limnohabitans* species is driven by its specific requirements. Vojtěch's merit is that he could define such requirements for the whole RBT lineage (bacteria targeted by the R-BT065 probe) of the *Limnohabitans* genus. Thereby, the relative abundance and absolute abundance of these bacteria were significantly and positively related to higher pH, conductivity and the proportion of low-molecular-weight compounds in DOC, and negatively related to the total DOC and dissolved aromatic carbon contents. Interestingly, members of the *Limnohabitans* genus exist in various microhabitats (i.e. patchy character of plankton, presence of organic particles, algae, cyanobacteria, protists, zooplankton associations etc.) selectively occupied by individual bacterial genotypes. The particle-like structures are important hot-spots of bacterial activity in oligotrophic pelagic waters, while interspecific networks (an addiction on different organisms) are present in all types of. Vojtěch's thesis suggests that the latter case is of particular importance for members of the *Limnohabitans* genus. The presented PhD thesis offers an excellent-model system to study unknown genomic traits, species diversification, environmental variables and interactions. Although recent approaches suggest a complex web structure of microbial interdependencies in aquatic systems, the present thesis adds to a better understanding of bacterial microdiversity and hence enables a better definition of relevant taxa.

Due to the high scientific relevance and the excellent performance of Vojtěch Kasalický, I rate his thesis as excellent (summa cum laude). I am convinced that Vojtěch has set a very well and promising basis for his future scientific career.



(Prof. Dr. Hans-Peter Grossart)

