MASARYKOVA UNIVERZITA

Annex No. 11 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

Habilitation Thesis Reviewer's Report

Masaryk University Faculty	Faculty of Science
Procedure field	Microbiology
Applicant	Mgr. Ivan Kushkevych, Ph.D., Dr.Sc.
Applicant's home unit, institution	Department of Experimental Biology, Faculty of Science at Masaryk University
Habilitation thesis	Functional role of sulfate-reducing bacteria in the development of bowel diseases in human and animals
Reviewer	John H. Cummings, MD, PhD, Professor of experimental gastroenterology
Reviewer's home unit, institution	Division of Cancer Research, Medical Research Institute School of Medicine at the University of Dundee (Scotland, United Kingdom)

The role of sulphate reducing bacteria (SRB) in the human intestine is an important but much neglected area of study. The central role of SRB in hydrogen metabolism in the gut, and in addition in soil and marine sediments, is critical in maintaining normal function, whilst at the same time the end-products of dissimilatory sulphate reduction such as hydrogen sulphide are highly toxic. I am therefore pleased that Dr. Kushkevych has chosen this area of study for his habilitation thesis.

The habilitation thesis opens with a comprehensive review of sulphate reducing bacteria, their various habitats, their characteristics both metabolic, microbiological, and genetic and their metabolism. This review is comprehensive and informative, up to date, well written and illustrated. It provides an excellent overview of SRB and will be of value to anyone wanting to learn about these bacteria and their role in nature. The wide range of substrates used by SRB is important to recognize, as is the variable carriage rate of these species across different populations of the world. The observation that oral sulphate supplements suppress methanogenesis in humans was I think first described by Christl in 1992. The section on hydrogen metabolism in the gut is a good summary of what is known on this subject. I note that much of the literature on the subject dates from around 20 years ago. It is a pity that this important area of gut physiology, with such implications for health, has not received more attention. This makes the habilitation thesis all the more important and Dr. Kushkevych is to be congratulated for pursuing his studies in this area over many years.

The co-existence of SRB with other microorganisms in various environments is well described. I was most interested to note the role of SRB at the junction between aerobic and anaerobic layers in marine sediments, where sulphate is in abundance. The importance of SRB as electron acceptor and the role of oxygen in detoxifying the products of metabolism in this unique ecosystem is an important point to make. This discussion leads naturally to a consideration of what is happening at the mucosal surface in the large intestine. Whilst the microflora of the mucosal surface is largely the same as those in the lumen the potential for more oxygen to be present is important. Dr. Kushkevych gives a useful overview of

fermentation reactions in these consortia and the likely interactions with the gut epithelium. This is clearly important in considering the aetiology of inflammatory bowel diseases. In this context it is worth remembering that sulphur amino acids are a significant source of sulphide in the large bowel (Magee American Journal of Clinical Nutrition, 2000 72:1488-1494.) and that meat has been linked to the cause of large bowel cancer. However experimental evidence for this link is difficult to obtain whilst that relating to ulcerative colitis in more readily available. Dr. Kushkevych makes a good case for SRB/sulphide being involved in the inflammatory process with evidence from animal studies and human observational epidemiology. Why colitis should always start in the rectum is something that I have always found puzzling.

I am pleased to see that Dr. Kushkevych has done some studies of the human gut flora in both healthy and colitis patients. The characteristic pattern of bacteria in colitis, high SRB and low 'protective' bacteria such as *Bifidobacteria* and *Lactobacilli* is important to note and should be of interest to the manufacturers of probiotic products. The higher concentrations of sulphide in colitis faeces would support these findings but should be interpreted only in the knowledge of what diets these people were eating. I am not competent to comment on the 'Isolation and identification of intestinal sulfate-reducing bacteria', which requires a detailed knowledge of these techniques for growing SRB etc. but it is reassuring to note that all the species isolated were members of the *Desulfovibrio* and *Desulfomicrobium* genera confirmed by 16S rRNA analysis. I am interested to learn about *D. piger*. The use of cluster analysis and various cross-correlation analyses is impressive and reassuring to know that 'the mechanisms of sulfate reduction process in intestinal SRB of different genera are almost identical'. This work uses state-of–the-art techniques to give us valuable information about SRB in the gut and their potential to cause disease.

I am particularly pleased that Dr. Kushkevych has given equal weight to the presence of inorganic sulphate and sulphite in the human diet. Sulphite is widely used by the food industry as a preservative especially in beer, wine, processed and preserved/dried food. This source is available for reduction in the anaerobic environment of the hindgut where the production of hydrogen sulphide is a potentially toxic by-product. This is an issue that the food industry has not yet fully addressed, but should do so. The two strains chosen for study *D. piger* Vib-7 and *D. orale* Rod-9 are not familiar to me but I note they have been isolated from the human gut. The differences in sodium and potassium transport and ATPase activity between these two strains in interesting but in the physiological environment of the gut may not be limiting. The temperature optima are close to physiological.

The kinetic parameters of dissimilatory ATP sulfurylase activity and differences are shown between the two chosen bacteria. The increase in sulphite reductase activity with increasing concentrations of sulphide is well within parameters found in the gut and levels that might be considered toxic to epithelial cells. The high production rate by some of these *Desulfovibrio* species leads to concern about their presence and activity in the human gut. The electron donors are well described.

Dr. Kushkevych the goes on to describe the basic physiology of *D piger* strain Vib-7. The effect of change in pH, concentration of accumulated hydrogen sulfide, concentration of electron acceptor/donor and the total number of SRB in the gut on the growth and metabolism of *D. piger* are important fundamental information about this strain.

Of particular interest to me as a gastroenterologist is the development of animal models of ulcerative colitis to help explore the role of SRB and their metabolites, principally hydrogen sulphide, in causing inflammation in the colonic mucosa. The cellular toxicity of hydrogen sulphide is well reviewed here, its ready diffusion into epithelial cells and

importantly the protective role of butyrate, first described by Roediger. Clearly diet will have a major role to play in providing substrates e.g.various carbohydrates, through fermentation for the production of protective factors along with the substrates e.g. s-amino acids, inorganic sulphate and sulphite and small amounts of SO_4 present as active groups in some polymers, required for sulphide production. The DNA damaging role of sulphide has received much less important and I am pleased to see it mentioned here. The intracellular detoxification of suphide by Rhodanase and Thiol S-methyltransferase is critical to our understanding of the fate of sulphide intracellularly. The changes in the rat gut microflora by the three diets are intriguing. In his conclusions Dr. Kushkevych is correct in arguing that we should pay attention to the gut microflora when trying to understand the pathogenesis of ulcerative colitis. In animal models no bacteria equals no inflammation. Using SRB and its products as biomarkers of risk and treatment is worth pursuing although many laboratories will not have the facilities to do this and inflammatory markers are better established. And certainly trying to identify suitable antimicrobials should be possible. The point that Dr. Kushkevych makes about 'the number of *Lactobacillus* and *Bifidobacterium* is reduced by 6–7 orders' is also very important and may open up other avenues to management and prevention. *D. piger* may have a role in this context.

In conclusion I am impressed by the high quality, originality and range of studies that Dr. Kushkevych reports together with his understanding of the importance of sulphate-reducing bacteria in nature and especially in the hindgut. I agree that these results will be of interest to medical and veterinary science as well as the community of nutritionists. The habilitation thesis is very well presented and is an important contribution to the subject. In addition I hope that Dr. Kushkevych will report more of his studies in the scientific literature. I strongly recommend awarding and assigning to Dr. **Ivan Kushkevych** by title "**Associate Professor**"

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)
I have no questions for the habilitation thesis defence.

Conclusion
The habilitation thesis entitled "**Functional role of sulfate-reducing bacteria in the development of bowel diseases in human and animals**" by Mgr. **Ivan Kushkevych**, Ph.D., Dr.Sc. **without a doubt fulfils** requirements expected of a habilitation thesis in the field of **Microbiology**.

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