## THE BIOLOGICAL INTERPRETATION OF METABOLOMIC DATA CAN BE MISLED BY THE EXTRACTION METHOD USED

S. G. Villas-Boas<sup>1</sup>, X. Duportet<sup>2</sup>, S. Carneiro<sup>3</sup>, R. B.M. Aggio<sup>1</sup>

<sup>1</sup>School of Biological Sciences, University of Auckland, Auckland, New Zealand <sup>2</sup>16 rue Claude Bernard, AgroParisTech, Paris, France <sup>3</sup>Institute for Biotechnology and Bioengineering, University of Minho, Braga, Portugal

The field of metabolomics is getting more and more popular and a wide range of different sample preparation procedures are in use by different laboratories. Chemical extraction methods using one or more organic solvents as the extraction agent are the most commonly used approach to extract intracellular metabolites and generate metabolite profiles. Metabolite profiles are the scaffold supporting the biological interpretation in metabolomics. Therefore, we aimed to address the following fundamental question: can we obtain similar metabolomic results and, consequently, reach the same biological interpretation by using different protocols for extraction of intracellular metabolites? We have used four different methods for extraction of intracellular metabolites using four different microbial cell types (Gram negative bacterium, Gram positive bacterium, yeast, and a filamentous fungus). All the samples were identical. After extraction and GC-MS analysis of metabolites, we did not only detect different numbers of compounds depending on the extraction method used and regardless of the cell type tested, but we also obtained distinct metabolite levels for the compounds commonly detected by all methods (p-value < 0.001). These differences between methods resulted in contradictory biological interpretation regarding the activity of different metabolic pathways. Therefore, our results show that different solvent-based extraction methods can yield significantly different metabolite profiles, which impact substantially in the biological interpretation of metabolomics data. Thus, development of alternative extraction protocols and, most importantly, standardization of sample preparation methods for metabolomics should be seriously pursued by the scientific community.