Development of proteomic methods on milk for ruminant phenotyping

Mylène Delosière, Isabelle Cassar-Malek, Laurence Bernard, Arnaud Delavaud, Didier Viala, Muriel Bonnet

Development of proteomic methods on milk for ruminant phenotyping

Mylène Delosière[1], Isabelle Cassar-Malek[1], Laurence Bernard[1], Arnaud Delavaud[1], Didier Viala[1,2], Muriel Bonnet[1].

[1] Université Clermont Auvergne, INRA, VetAgro Sup, UMR1213 Herbivores, F-63122 Saint-Genes-Champanelle, France.
[2] INRA, Plateforme d'Exploration du Métabolisme composante Protéomique, F-63122 Saint-Genès-Champanelle, France.

A challenge for livestock production is to improve animal performances and the quality and safety of their products while ensuring welfare and health [1]. To face this challenge, tools are needed for assessing rapidly, efficiently and cheaply the multiple traits of performances. Proteomic screening of milk provides a good opportunity for discovering biomarkers to be adopted in such tools. Therefore, we aim at developping rapid and affordable proteomics of milk for dairy ruminants' phenotyping.
Raw milk is a complex fluid requiring fractionation techniques in order to enhance protein characterization. As a first step in milk fractions analysis, we focused on the subproteome of the milk fat globules membranes (MFGM). Two MFGM protein purification protocols were applied to fresh milk from Holstein cows. MFGM were Tris HCl-SDS washed during 1h, then proteins treated with acetone during 20 h [2] (**ACE**) or MFGM were cristallized at 4°C overnight to harden the fat globules [3] (**CRI**). Prior to nano LC-MS/MS analysis, two digestion protocols were tested: 1/ in-solution digestion (**SD**) and 2/ in-gel digestion after a concentration of proteins in one band stacking gel (**GD**).
The combination of all methods allowed identifying 218 unique proteins. From CRI extraction, we identified 179 and 65 proteins after GD and SD, respectively, but only 93 and 31 proteins from ACE extraction. Comparable results were obtained on frozen milk. The greatest protein identification with CRI compared to ACE extraction could be due to a higher preservation of proteins using cold cristallisation than aggressive acetone. GD allowed more protein identifications probably due to a higher lipid elimination compared to SD. A total of 88 proteins were specific of the combination of CRI extraction and GD protocol; this combination will be used for future investigation of the MFGM proteome. Similar developments are still under progress on the whey fraction.

(1) Roncada, P., et al. (2012). "Farm animal milk proteomics." Journal of Proteomics **75**(14): 4259-4274.
(2) Ji, X. X., et al. (2017). "Differences in proteomic profiles of milk fat globule membrane in yak and cow milk." Food Chemistry **221**: 1822-1827.
(3) Yang, Y., et al. (2015). "Proteomic characterization and comparison of mammalian milk fat globule proteomes by iTRAQ analysis." Journal of Proteomics **116**: 34-43.