*Citrobacter rodentium*感染マウス大腸のイオンモビリティーを利用したラベルフリーLC/MS解析

佐藤 太[1], Hughes Chris[2], Vissers Johannes[2], Collins James[3], Frankel Gad[3]
[1]日本ウォーターズ株式会社; [2]Waters Corporation; [3]Imperial College

INTRODUCTION

*Citrobacter rodentium*はマウス特有の病原菌で、腸管病原性および腸管出血性大腸菌のヒトへの感染の研究に用いられる。*C. rodentium*はA/E障害(attaching and effacing lesion)の形成を介して大腸にコロニーを形成し、C57BL/6マウスに伝染性大腸過形成および自己限定性疾患を引き起こす。

本研究では、感染の2つの重要な時点、8日目(細菌のコロニー形成のピーク)および14日目 (大腸過形成のピーク)のマウスの大腸のプロテオームの変化を決定するために定量プロテオーム解析を行った。

大腸プロテオームの相対的な存在量の変化を決定するためにイオンモビリティおよび質量分析器と組み合わせた液体クロマトグラフィーを用いた。続く解析では、マウス大腸におけるタンパク質発現において、鍵となる病原性決定因子の細菌変異体の影響に焦点を当てた。

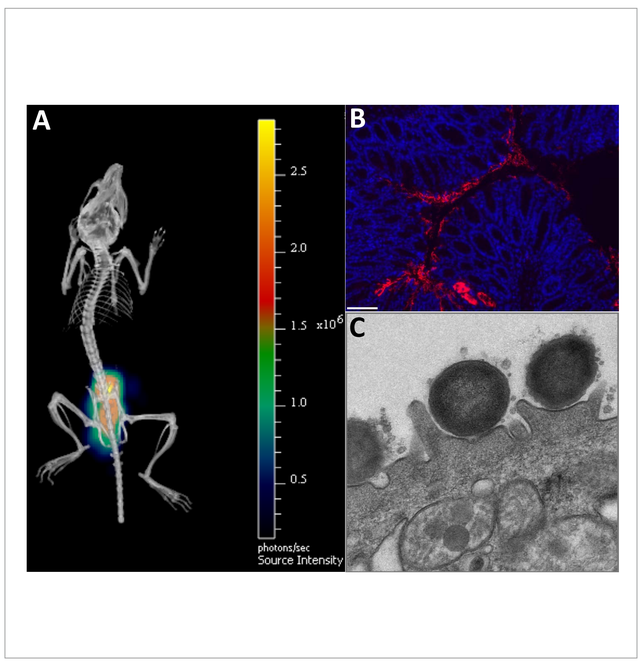

Figure 1. Murine C. rodentium infection. A) DLIT-µCT image of bioluminescent C. rodentium colonizing the colon of a mouse at day 8 post infection, B) Indirect immunofluorescence assay of C. rodentium (Red) adherent to the colonic mucosa (depicted by blue cell nuclei), C) Transmission electron microscopy of C. rodentium attaching and effacing lesions.

METHODS

Sample preparation

Six week old C57 Bl/6 mice were infected, as illustrated in Figure 1, with *C. rodentium* or administered PBS as a control. At day 8, or day 14 post infection, mice (4 infected, 4 control) were euthanized, 6 cm of colon removed and pooled (2 colons per sample) for protein extraction and trypsin digestion.

LC-MS conditions

All samples were analyzed in triplicate by LC-MS using a nanoACQUITY system coupled to a Synapt G2-S mass spectrometer, Figure 2. Samples were injected onto a 5 µm Symmetry C18 180 µm x 20 mm trapping column for 5 min at 15 µL/min with 0.1% (v/v) aqueous formic acid. Peptides were separated on a 1.8 µm HSS T3 75 µm x 150 mm analytical column using a gradient of 99% A (0.1% (v/v) formic acid) 1% B (99.9% acetonitrile 0.1% (v/v) formic acid) to 40% B over 90 min at 300 nL/min. Column eluent was then coupled to an nanoelectrospray emitter and analysed in the mass spectrometer using ion mobility (IM) assisted label-free data independent analysis (DIA), as illustrated in Figure 3.

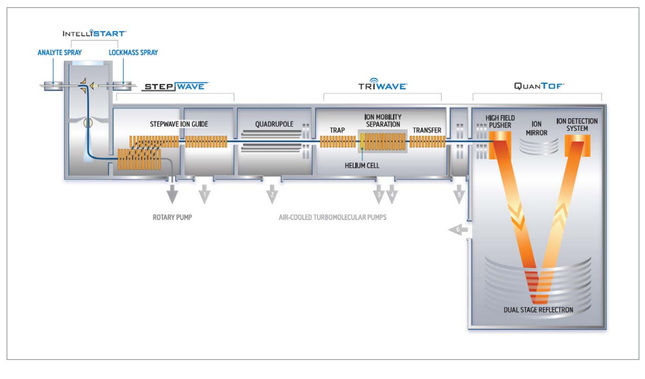

Figure 2. Synapt G2-S Ion Mobility Enabled Mass Spectrometer. The Ion Mobility Separation is performed in the TriWave region.

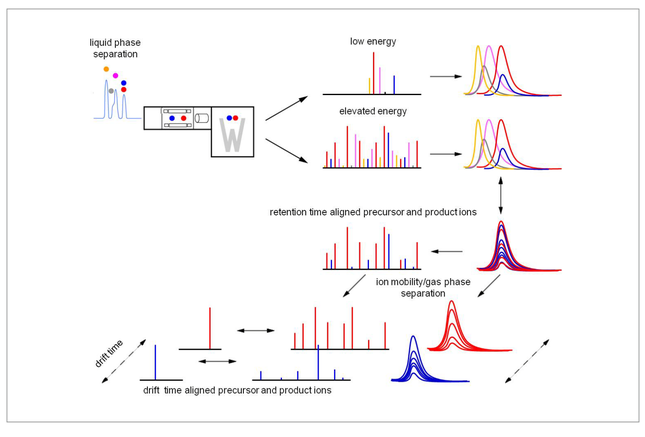

Figure 3. Label-free LC-IM-DIA-MS (HDMS^E) scanning method.

Data analysis

Data was processed and searched against a non redundant species specific UniProt database with ProteinLynx GlobalSERVER v2.5.2. Further interrogation of the dataset allowed for the calculation of relative within-sample abundances for each identified protein, forming the input for the quantitative/qualitative and cluster analysis of the data using SIMCA P+ and/or Spotfire.

RESULTS

Analytical precision and accuracy

Some example peptides, show in Figure 4, were monitored to assess LC retention time and IM drift time reproducibility. For the shown peptide, RSD values (n = 24) of 0.9% and 0.6%, were obtained, respectively. Mass measurement accuracy, based on the database search results, was typically better than 2 ppm. This type of consistency was observed for the complete label-free LC-MS data set.

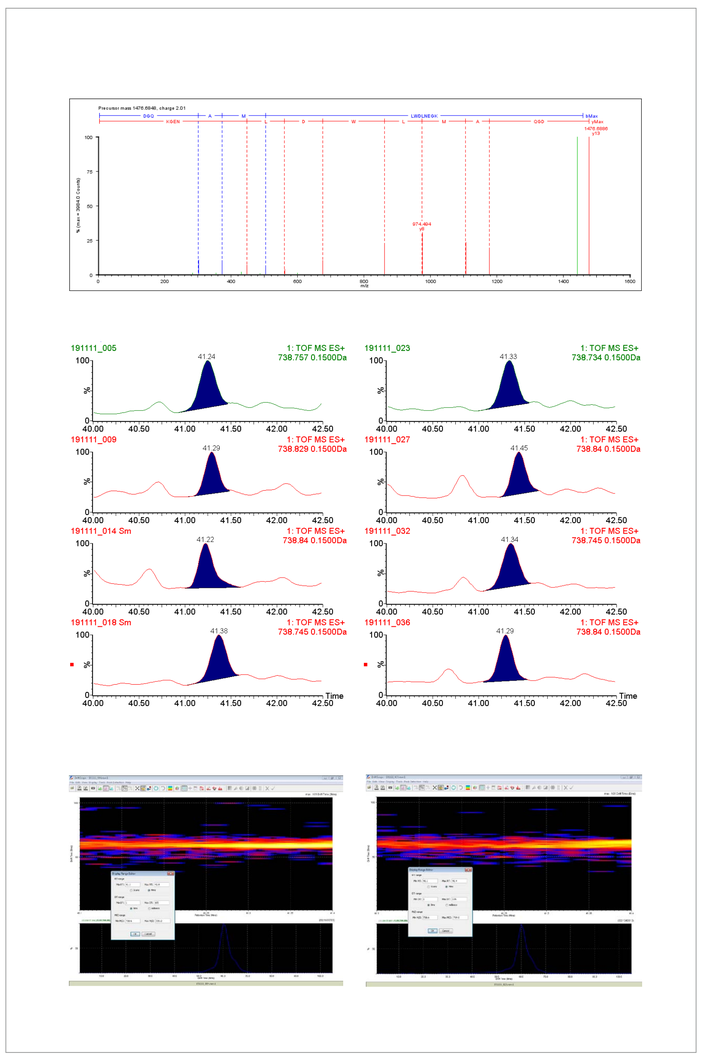

Figure 4. Extracted mass chromatogram LC-MS (middle) and drift time (bottom) profiles DGQAMLWDLNEGK GBLP_MOUSE.

Qualitative and quantitative analysis

The LC-MS data were normalized by expressing the estimated protein amount as a function of the total detected amount per injection. Average were calculated for the technical replicates and not attempts made to remove statistical outliers. Unsupervised principal component analysis (PCA) revealed that classification of the data was feasible on sample type, *i.e.* uninfected *vs.* infected, and time of infection as illustrated in Figure 5.

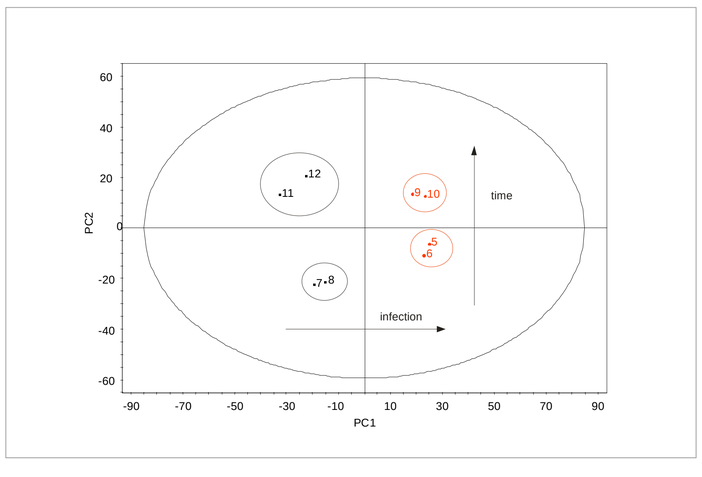

Figure 5. Unsupervised PCA ^{10}log converted within sample amounts; infected (black), uninfected (red).

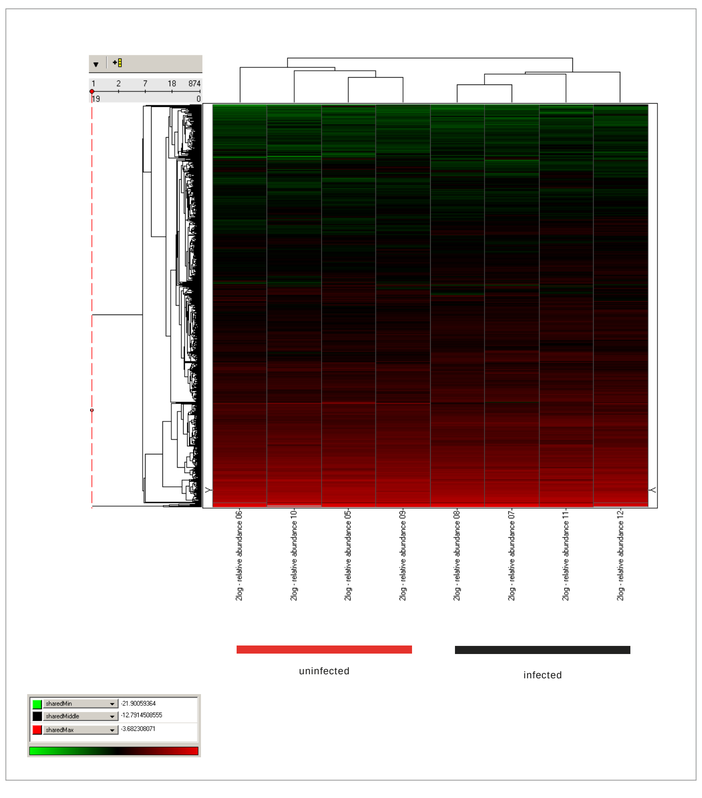

Figure 6. Hierarchical clustering subset ^{2}log converted within sample amounts.

A subset of the data, in order to prevent bias during analysis, was subjected to hierarchical clustering. The results, shown in Figure 6, also illustrate that classification of the results was feasible using an alternative clustering method.

Further grouping of proteins illustrating similar regulation trends was conducted by K-means clustering of which an example is shown in Figure 7, including the statistical distribution of one of the proteins of interest. An excerpt of the clustering and grouping is provided in Figure 8, showing the regulation and measurement variation of selected proteins of interest. A validation example is shown in Figure 9.

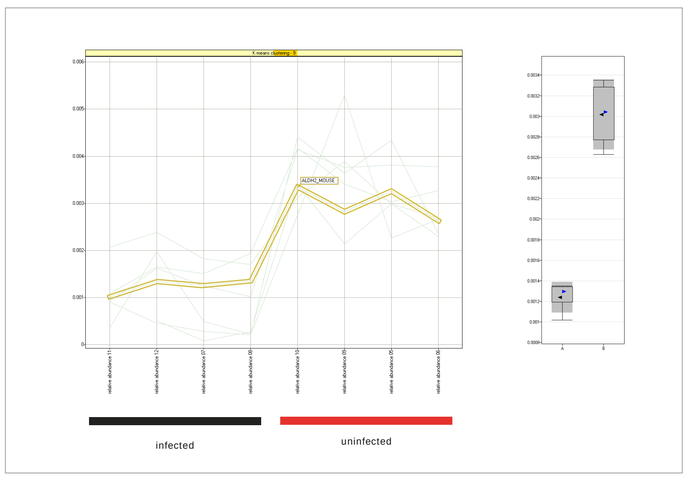

Figure 7. K-means clustering result z-score converted within sample amounts of a subset of proteins showing significant down-regulation following infection and statistical distribution (box-and-whisker; A = infected, B = uninfected) of ALDH2.

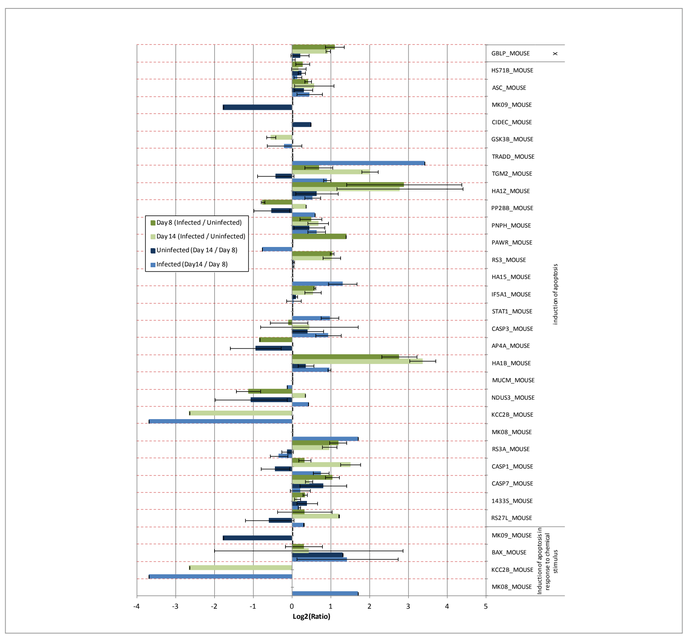

Figure 8. Selected protein amount distribution and measurement variation examples as functions of time and infection.

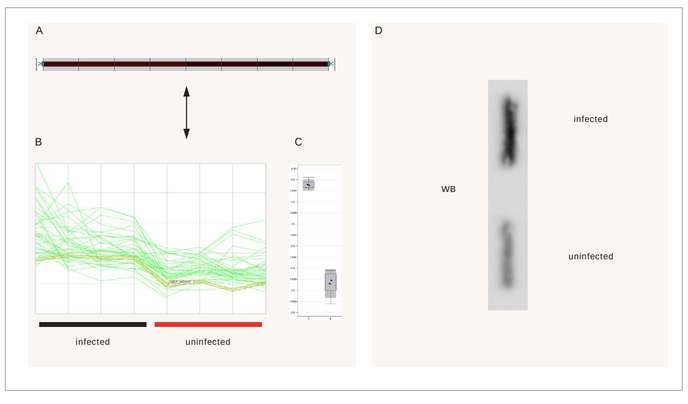

Figure 9. Label-free LC-IM-DIA-MS quantitation GBLP vs. blotting. A) hierarchical clustering, B) trend plot and C)statistical distribution (A = infected, B = uninfected)LC-MS results, and D) Western blot data.

CONCLUSIONS

- **サンプルセット全体から、1つ以上のユニークなペプチドから成る2852のタンパク質が同定された。**
- **得られたデータの詳細な解析により、試料内の同定されたタンパク質の相対量が算出され、発現の傾向が一致するタンパク質をグルーピングすることができた。**
- **相対存在量の計算値の教師なし階層的クラスタリングにより、*C. rodentium* 感染マウスおよび非感染マウスが明確に分類され、ホストと病原性両方の感染症関連タンパク質の同定が可能となった。**

References

1. Citrobacter rodentium of mice and man. Mundy R, MacDonald TT, Dougan G, Frankel G, Wiles S. Cell Microbiol. 2005 Dec;7(12):1697-706.
2. In vivo bioluminescence imaging of the murine pathogen Citrobacter rodentium. Wiles S, Pickard KM, Peng K, MacDonald TT, Frankel G. Infect Immun. 2006 Sep;74(9):5391-6.
3. Absolute quantification of proteins by LCMSE: a virtue of parallel MS acquisition. Silva JC, Gorenstein MV, Li GZ, Vissers JP, Geromanos SJ. Mol Cell Proteomics. 2006 Jan;5(1):144-56.

Acknowledgements

Gushinder Kaur-Atwal, James Wright and Jyoti Choudhary are kindly acknowledged for their valuable contributions throughout the development of this work.

MKT12095