Supplementary Information

Rapid differentiation of *Campylobacter jejuni* cell wall mutants using Raman spectroscopy, SERS and mass spectrometry combined with chemometrics

Malama Chisanga,[a] Dennis Linton,[b] Howbeer Muhamadali,[c] David I. Ellis,[a] Richard L. Kimber,[d] Aleksandr Mironov,[b] and Royston Goodacre*[c]

[a]School of Chemistry, Manchester Institute of Biotechnology, University of Manchester, Manchester, UK, M1 7DN

[b]School of Biological Sciences, Faculty of Life Sciences, University of Manchester, Manchester, UK

[c]Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Liverpool, UK, L69 7ZB

[d]School of Earth and Environmental Sciences, University of Manchester, Manchester, UK, M13 9PL

*Corresponding author:
Royston Goodacre, E-mail: roy.goodacre@liverpool.ac.uk, Tel: 0151 795 7689

Keywords: Raman spectroscopy, SERS, silver nanoparticles, MALDI-TOF MS, multivariate analysis, *Campylobacter jejuni*, mutants, microbial differentiation

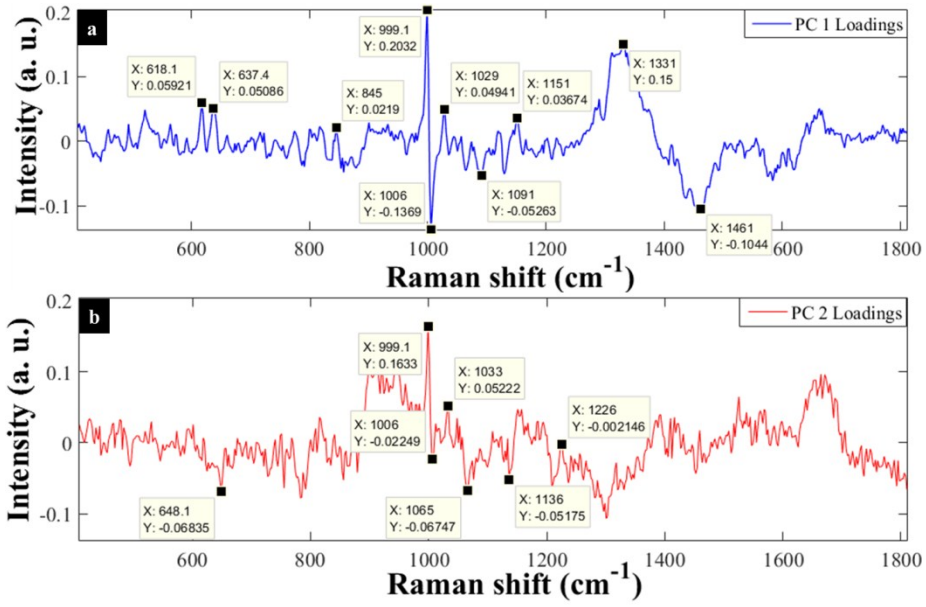

Figure S1. PC1 **(a)** and PC2 **(b)** loadings for Raman spectral data of the *Kps* mutants: *KpsM*–, *KpsS*– and *KpsC*–. The numbers on respective spectral peaks represent wavenumbers of the main explanatory input variables responsible for the clustering trends observed on PCA scores in Figure 6a.

Supplementary Information

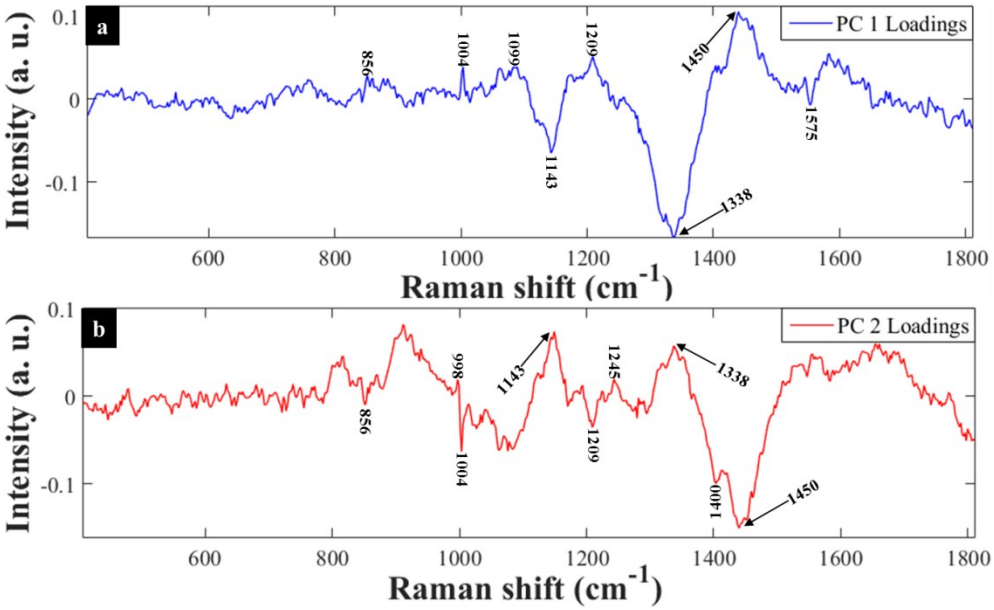

Figure S2. PC1 **(a)** and PC2 **(b)** loadings plot for Raman spectral data of all *C. jejuni* and mutants examined in this study. The numbers on respective peaks represent wavenumbers of the major explanatory spectral input variables associated with the separation patterns of clusters on PCA scores in Figure 4a.

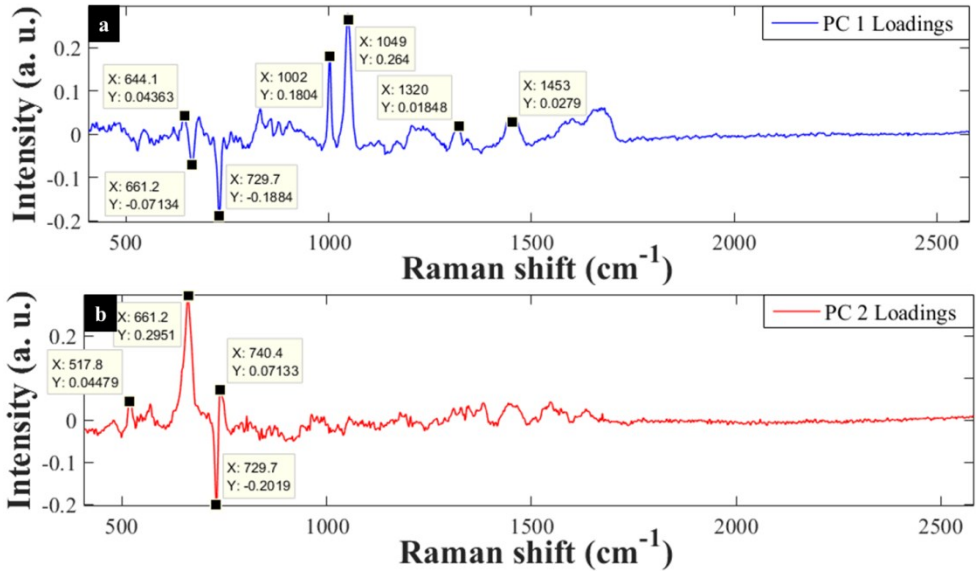

Figure S3. PC1 **(a)** and PC2 **(b)** loadings plots for *in situ* external SERS spectral data of the three *Kps* mutants: *KpsM*$^{-}$, *KpsS*$^{-}$ and *KpsC*$^{-}$. The numbers on respective spectral vibrations represent wavenumbers of significant spectral inputs linked to the clustering patterns on PCA scores in Figure 6b.

Supplementary Information

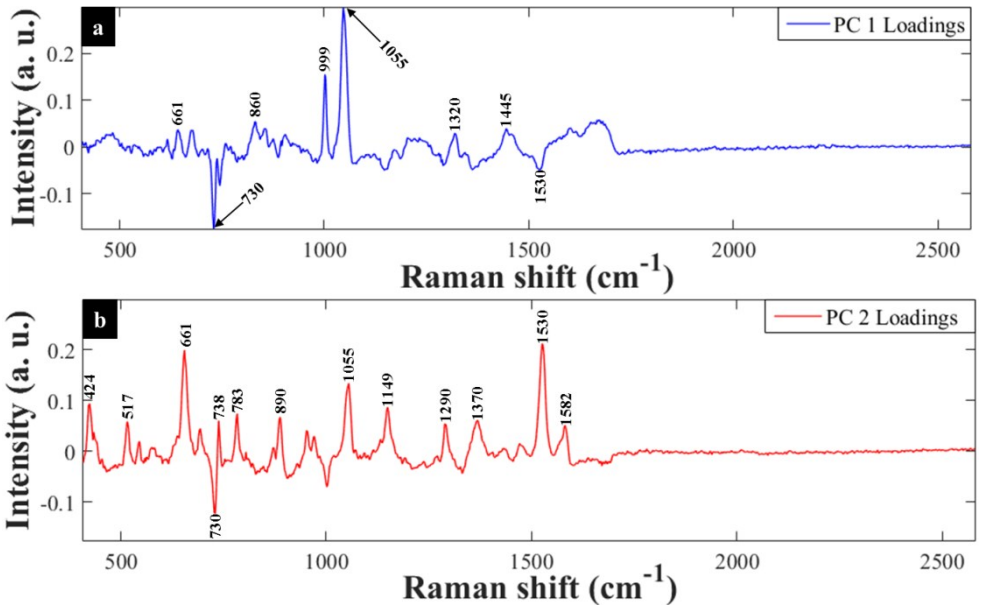

Figure S4. PC1 **(a)** and PC2 **(b)** loadings plots for *in situ* external SERS data of *C. jejuni* and derivative mutants generated from SERS spectra in Figure 3b. The numbers on respective peaks represent wavenumbers for the significant spectral input variables associated with the distribution of clusters on PCA in Figure 4b.

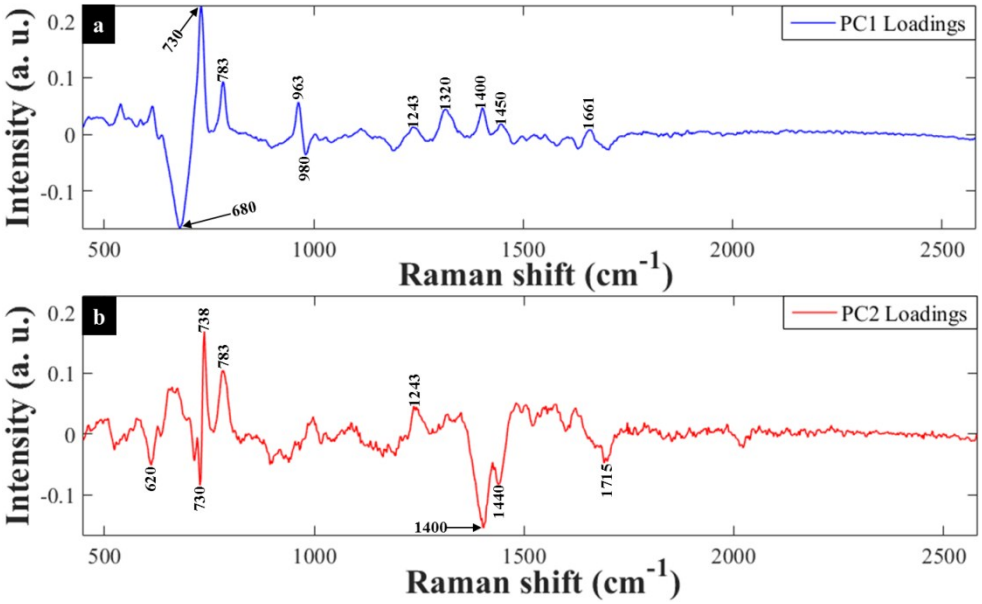

Figure S5. PC1 **(a)** and PC2 **(b)** loadings plot for *simple mixing* SERS data of three *Kps* mutants: *KpsM*$^{-}$, *KpsS*$^{-}$ and *KpsC*$^{-}$. The numbers on each peak represent wavenumbers of the major explanatory inputs responsible for the clustering trends observed on PCA scores in Figure 6c.

Supplementary Information

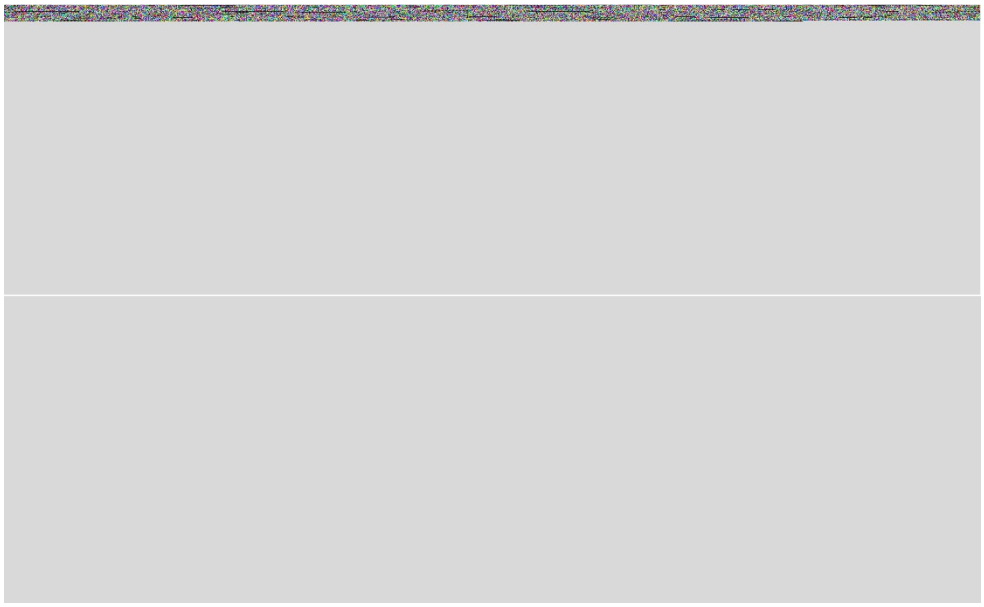

Figure S6. PC1 **(a)** and PC2 **(b)** loadings plots for *simple mixing* SERS spectral data of *C. jejuni* and mutants generated from SERS spectra in Figure 3c. The numbers on the spectral bands indicate wavenumbers of the major spectral inputs responsible for the clustering patterns on PCA scores in Figure 4c.

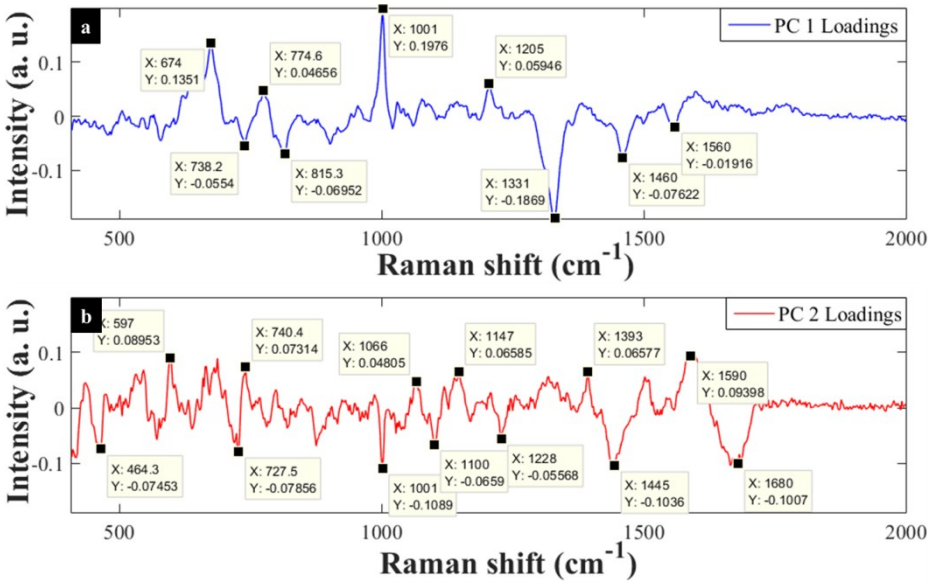

Figure S7. PC1 **(a)** and PC2 **(b)** loadings plot for *in situ* internal SERS spectra of three *Kps* mutants: *KpsM*$^{-}$, *KpsS*$^{-}$ and *KpsC*$^{-}$. The numbers on bands represent wavenumbers for the most important spectral input variables associated with the clustering patterns on PCA scores in Figure 6d.

Supplementary Information

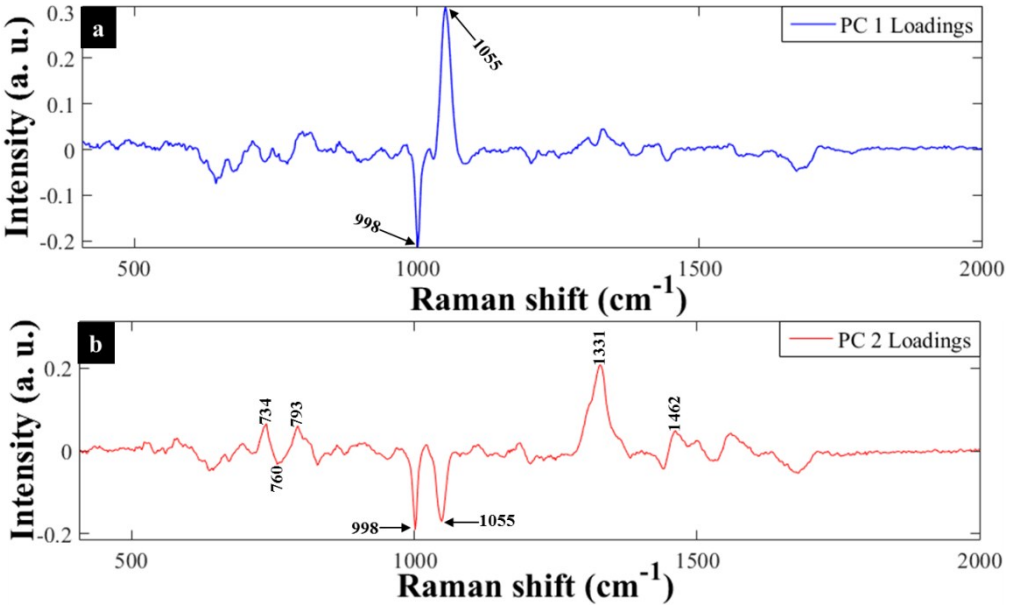

Figure S8. PC1 **(a)** and PC2 **(b)** loadings plots for *in situ* internal SERS spectral data of *C. jejuni* strain and mutants tested in this study generated from SERS spectra in Figure 3d. The numbers on the peaks represent wavenumbers for the main explanatory spectral variables responsible for the clustering trends observed on PCA scores in Figure 4d.

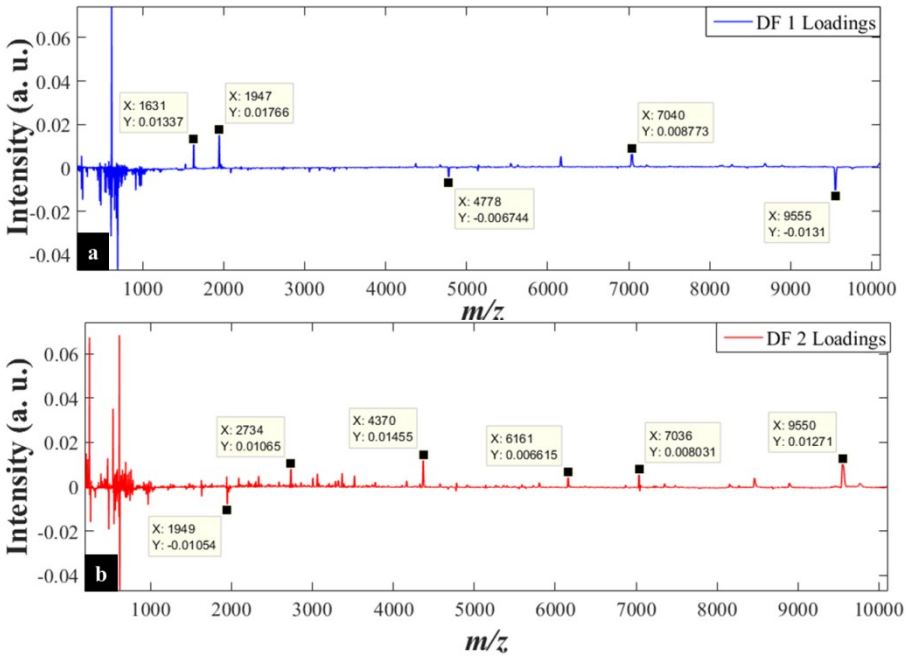

Figure S9. DF1 **(a)** and DF2 **(b)** loadings plots for MALDI-TOF-MS spectral data of the *Kps* mutants. The numbers on respective spectral peaks represent *m/z* for the significant variables linked with the clustering trends observed on DFA scores in Figure 6e.

Supplementary Information

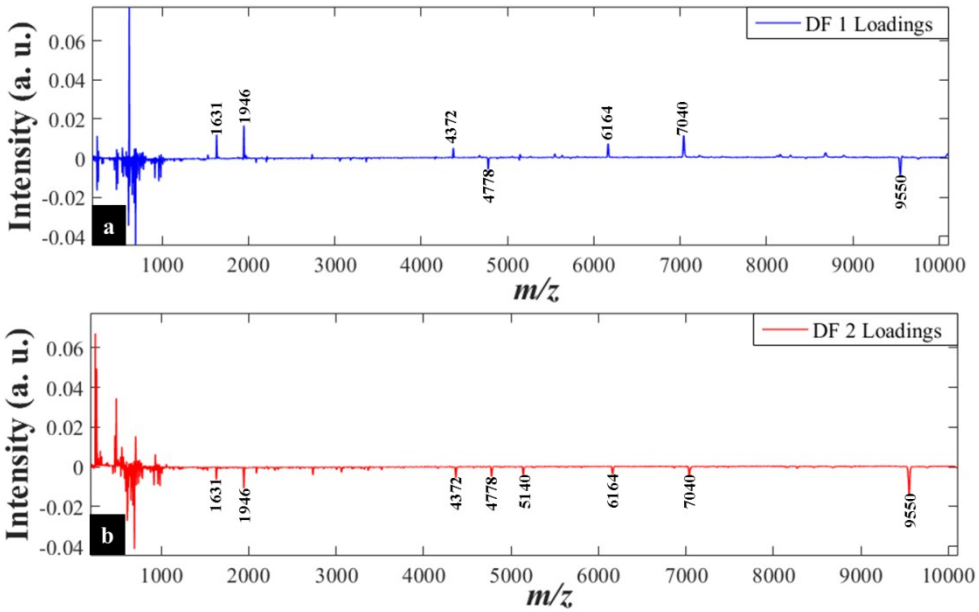

Figure S10. DF1 **(a)** and DF2 **(b)** loadings plots of MALDI-TOF-MS data for all bacteria obtained via MALDI-TOF-MS spectra in Figure 3e. The numbers on the respective peaks represent mass-to-charge ratios (*m/z*) for the main explanatory spectral input variables associated with DFA scores on Figure 4e.