**Electronic Supplementary Information for**

# Differentiation of Microbial Species and Strains in Coculture Biofilms by Multivariate Analysis of Laser Desorption Postionization Mass Spectra

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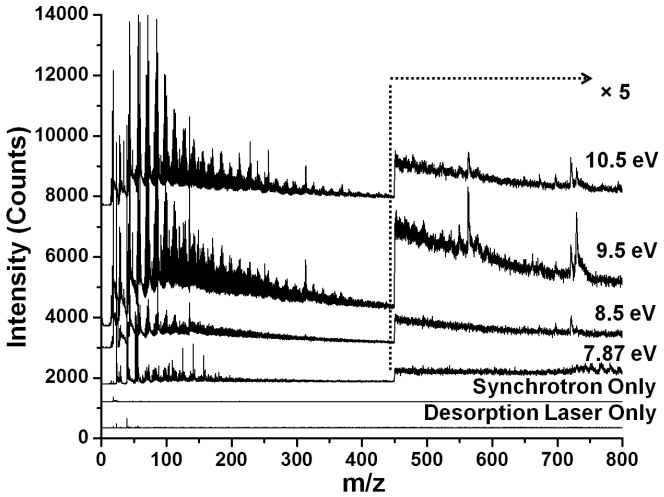


Figure S1: 7.87 to 10.5 eV synchrotron LDPI-MS of *E. coli* (tomato) strain blotted monoculture biofilm. Bottom two traces show the controls performed to check for any background signal.

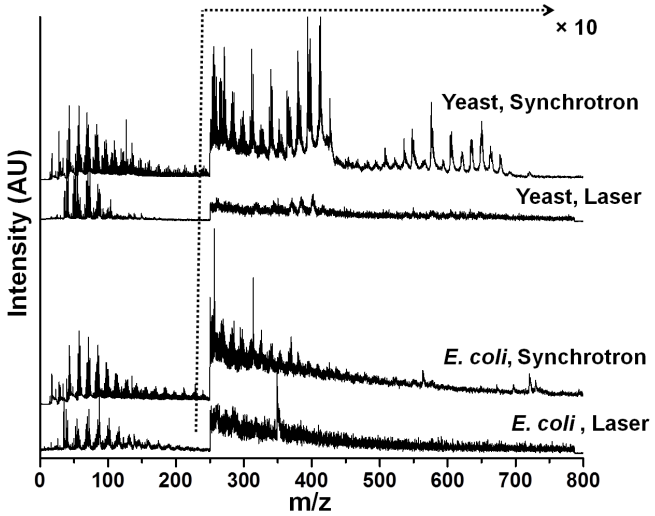


Figure S2: 10.5 eV laser and synchrotron LDPI-MS of blotted monoculture biofilms using two different instruments. *E. coli* (tomato strain) and yeast biofilms. Each spectrum in the figure is normalized to correct for differences in data acquisition strategies between the two instruments.

**Table S1**: List of mass peaks (m/z) first observed at 7.87, 9.5, and 10.5 eV photon energies by synchrotron LDPI-MS of blots of *E. coli* bacteria and *S. cerevisiae* yeast monocultures.

| Species | 7.87 eV (m/z) | 9.5 eV (m/z) | 10.5 eV (m/z) |
|---|---|---|---|
| ***Escherichia coli*** **(Bacteria)** | 39-45, 53-58, 65-75, 77-87 | 35 | |
| | 95-105, 108-115, 120-130, 135-145, 157, 159, 165, 175, 180, 181,197 | 153 | |
| | 267 | 212-216, 240, 251 | 228, 256 |
| | 366 | 315, 326, 338, 371 | |
| | 590 | 550, 565, 578 | |
| | 605, 625, 635 | | 665 |
| | 732, 745, 763, 780, 793 | 730, 750, 775 | |
| ***Saccharomyces cerevisiae*** **(Yeast)** | 38-45, 53-60, 67-75, 82-88 | | 32, 34, 48 |
| | 90-105, 105-115, 135-143 | 145-150 | 128, 156, 168-172, 184-194, 198-205 |
| | 235-245, 250-260, 264-270, 276-284 | 213-216, 227-230, 272 | |
| | 397 | 394, 398, 400 | 395, 396 |
| | 413 | 412, 415, 426 | 494 |
| | 576 | 508, 536, 564-568, 592-596 | 510-514, 522, 552 |
| | 696-700 | | |
| | 720-724 | | |
| | 816-818, 840-845 | | |

MS peaks first appearing at 7.87 eV are shown in the left column of Table S1 while additional peaks appearing at 9.5 and 10.5 eV photon energies are listed in the middle and right column, respectively. Peaks observed at lower photon energies always appeared at higher photon energies as well, albeit at higher intensities. However, a separate column is not shown for 8.5 eV photon energy, since no new peaks were observed visually beyond those already present at 7.87 eV.

Several metabolites were tentatively assigned to the observed MS peaks, which are listed in Table S1, based on the ability of 7.87 eV photon energy to selectively ionize species containing tertiary amines and fused ring structures. These tentative assignments were made by referring to *E. coli*[1] and yeast[2] metabolite databases. *E. coli* peaks at m/z 102, 104, 114, 138, 140, 143, 145, 157, and 366 were assigned to betaine aldehyde, choline, 2-mercapto-1 methylimidazole, urocanic acid, L-histidinal, crotonobetaine, gamma-butyrobetaine, imidazolelactic acid, and phosphoribosyl formamidocarboxamide, respectively. Yeast peaks at m/z 157, 240, 243, 244, 252, 267, 284, and 720 were assigned to N-acetyl-D-proline, anserine, cytidine, uridine, 2'-deoxyinosine, adenosine, xanthosine, and phosphoribosyl-ATP, respectively.

Additionally, *E. coli* peaks at m/z 625, 635, 732 and 763 and yeast peaks at m/z 723, 724, 840, and 843 correspond with the molecules in the glycerophospholipids class, an abundant constituent of the microbial cell membrane. These molecules do not have tertiary amines or fused ring moeities in their structure, leading to the speculation that they may have desorbed as clusters which facilitates their VUV postionization.[3]

Additional classes of molecules will be photoionized at 9.5 and 10.5 eV photon energies, increasing the possibility of the parent ions of different endogenous species appearing at the same nominal m/z values. Nonetheless, tentative assignments were made for some 9.5 and 10.5 eV peaks in Table 1. *E.coli* peaks at m/z 153, 228, 240, 256, and 338 could be attributed to to 3-sulfinoalanine, myristic acid, L-cystine, palmitic acid, and N5-carboxyaminoimidazole ribonucleotide, respectively. Peaks at m/z 665, 750 and 775 were assigned to the molecules of glycerophospholipids. Similarly, yeast peaks at m/z 146, 149, 189, 190, 192, 200, 213, and 214 were assigned to L-glutamine, D-methionine, N-acetyl-L-glutamic acid, oxalosuccinic acid, citric acid, lauric acid, 4-phopho-L-aspartic acid, and 3-methylbutyl octanoate, respectively.

Peaks at m/z 565, 567 and 593 were assigned to glycerolipids and peaks at m/z 426, 494 and 522 were assigned to glycerophospholipids. Regardless of photon energy, high resolution or tandem MS analysis is required to unequivocally associate any of the aforementioned peaks with specific endogenous species in the cocultures.

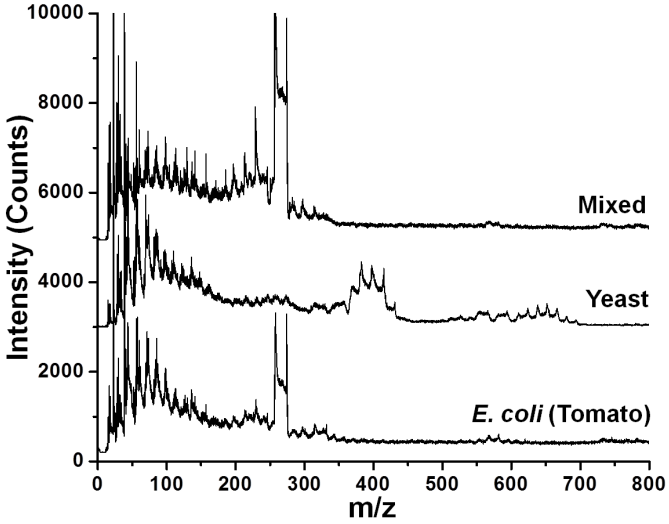


Figure S3: 10.5 eV synchrotron LDPI-MS of different regions of a blotted coculture biofilm. The distinct *E. coli* (tomato strain) and yeast regions as well as the "Mixed" overlapping region were examined.

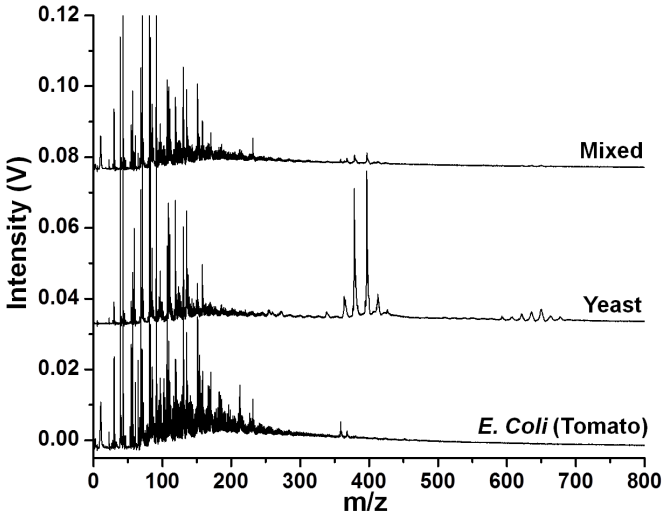


Figure S4: 7.87 eV laser LDPI-MS of three regions of *E. coli* (tomato strain) and yeast coculture membrane biofilm, including the "Mixed" overlapping region.

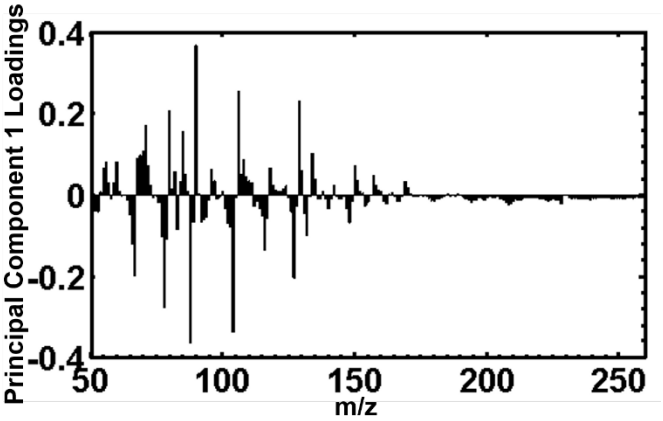


Figure S5: Principal component 1 loadings plot for the 7.87 eV laser LDPI-MS data set of the coculture multistrain biofilm.

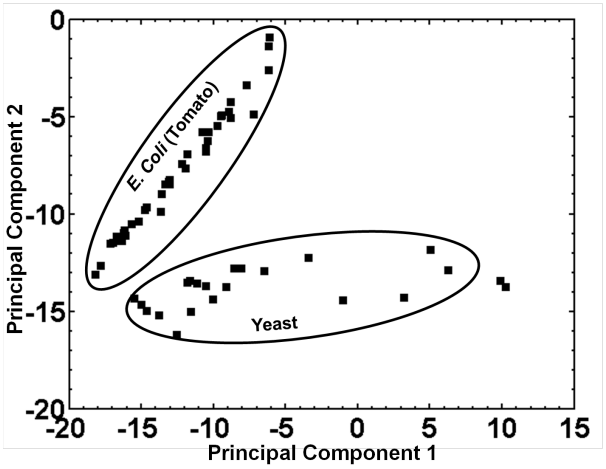


Figure S6: Principal component analysis of 10.5 eV laser LDPI-MS of a *E. coli* (tomato strain) and yeast coculture membrane biofilm, compared the regions of each biofilm that were far from the mixed region that defines the boundary between the two (referred to as "pure" in text).

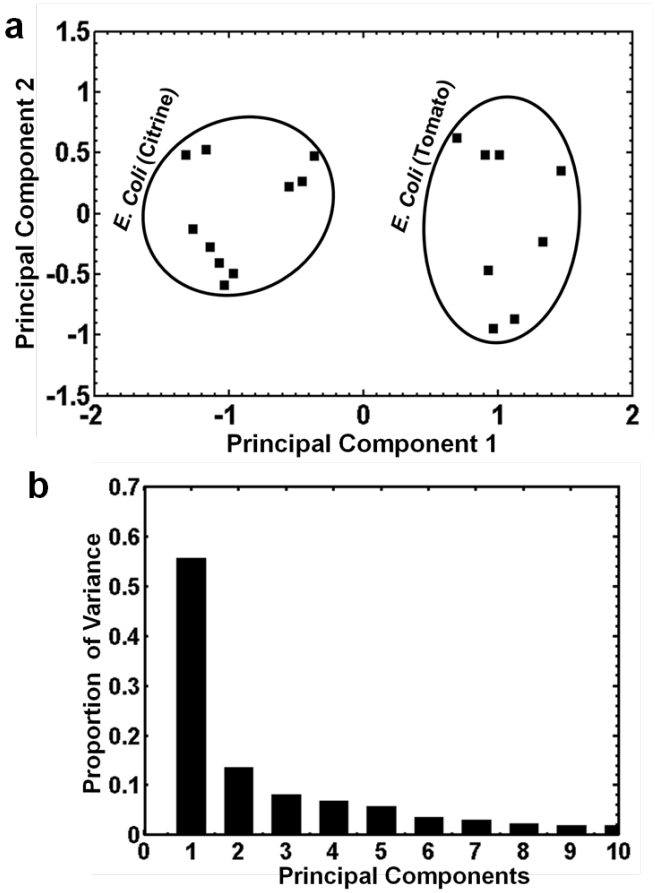


Figure S7: a) Principal component analysis of 10.5 eV laser LDPI-MS of a coculture tomato and citrine *E. coli* membrane biofilm. b) Scree plot showing the variance of the data with respect to the principal components.

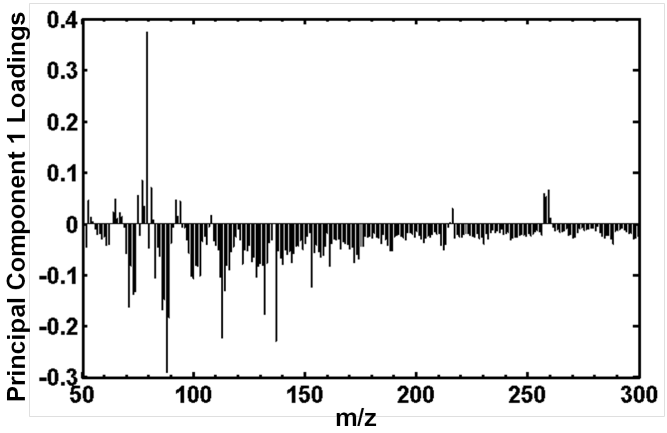


Figure S8: Principle component 1 loadings plot from 10.5 eV laser LDPI-MS data for coculture *E. coli* biofilm analyzed in Figure S7.

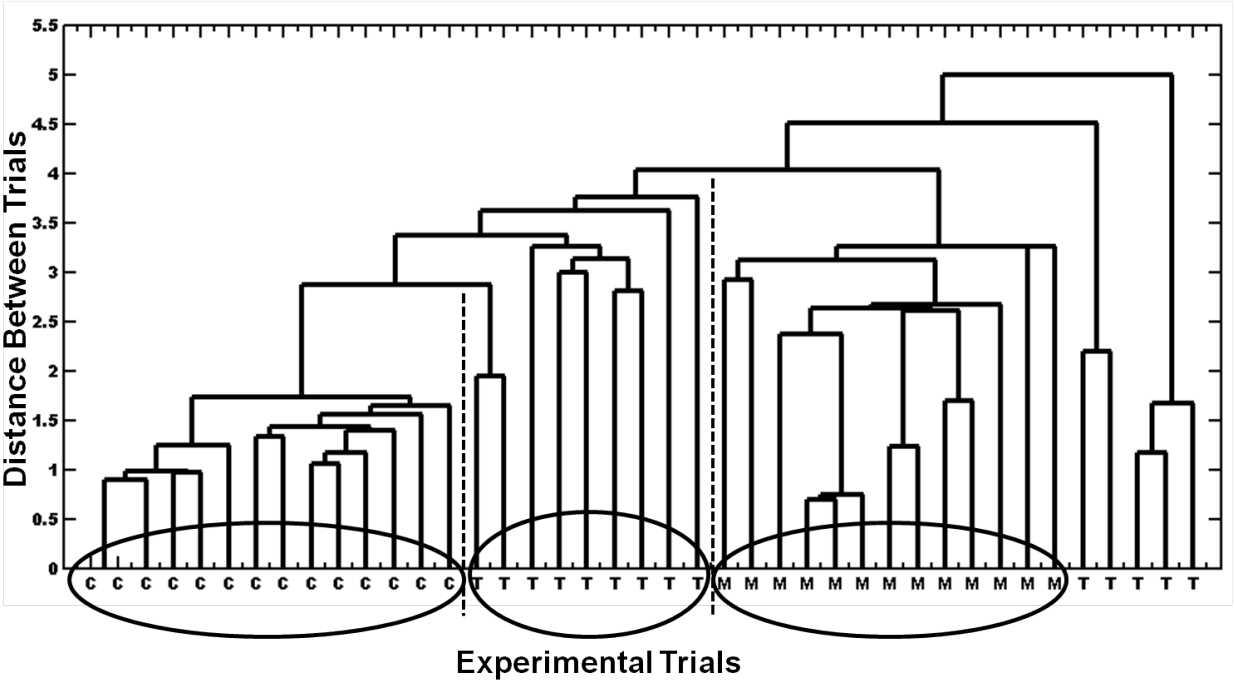


Figure S9: Hierarchical cluster analysis for 7.87 eV LDPI-MS data. The figure shows clustering of mixed region outside of the two pure strains. The y-axis shows the distance between the connected data sets and x-axis list the MS trials for each strain. In general, higher distance between trials indicates less correlated data.


1. A. C. Guo, T. Jewison, M. Wilson, Y. Liu, C. Knox, Y. Djoumbou, P. Lo, R. Mandal, R. Krishnamurthy and D. S. Wishart, *Nucl. Acids Res.*, 2013, **41**, D625-D630.
2. T. Jewison, V. Neveu, J. Lee, C. Knox, P. Liu, R. Mandal, R. K. Murthy, I. Sinelnikov, A. C. Guo, M. Wilson, Y. Djoumbou and D. S. Wishart, *Nucl. Acids Res.*, 2012, **40**, D815-D820.
3. M. Blaze M.T., L. K. Takahashi, J. Zhou, M. Ahmed, G. L. Gasper, F. D. Pleticha and L. Hanley, *Anal. Chem.*, 2011, **83**, 4962-4969.