

# Molecular and chemical dialogues in bacteria-protozoa interactions

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**Figure S1.** The number of genes that are up-regulated (Blue) or down-regulated (Red) in *P. fluorescens* SS101 cells exposed to *N. americana*. The genes are categorized into COGs A thru X (for specification of each of the COGs, see Table S1). Some genes can be placed in more than one COG and thus counted more than once.

**Figure S2.** MALDI imaging mass spectrometry (IMS) shows no production of massetolide A during the  $\Delta$ massA mutant-*N. americana* interaction. a.u. = arbitrary units.

**Figure S3.** Whole genome transcriptome analysis of *P. fluorescens* strains SS101 and SBW25 in the presence of *N. americana*. Heat maps showing log<sub>2</sub>-fold changes in the expression of genes that are differentially regulated in both SS101 (A) and SBW25 (B) upon protozoan grazing. Wild type SS101 and SBW25 were grown on 1/5 NBY plates at 25°C in the presence of *N. americana* for 2-3 days. Cells were collected and total RNA was extracted followed by cDNA synthesis, labelling and hybridization to a SS101/SBW25 whole-genome tiling microarray. The fold changes shown here represent averages of four biological replicates. For a list of all genes differentially regulated in SS101 and SBW25, we refer to Supplementary Tables S1 and S3.

**Figure S4.** (A) MALDI imaging mass spectrometry (IMS) shows production of viscosin and its derivatives during the *P. fluorescens* SBW25-*N. americana* interaction. a.u. = arbitrary units. (B) MS/MS network analysis and annotation of ion clusters from the *P. fluorescens* SBW25-*N. americana* interaction. Ion clusters in the black square represents the lipopeptide viscosin and its derivatives; the black circle represents the 325 m/z ion cluster; the grey square represents the 766 m/z ion cluster. (C) MS/MS analysis further indicated that the parent ion

with 1148.70 m/z detected in the *P. fluorescens* SBW25- *N. americana* interaction is most likely viscosin.

**Figure S5.** Box plots depicting the production of viscosin and its derivatives in *P. fluorescens* SBW25 alone, *N. americana* alone, *P. fluorescens* SBW25-*N. americana* interaction and *viscA* mutant alone. The box plots represent the median intensity in arbitrary units after TIC normalization (horizontal line), the upper and lower quartiles (box layout, spectra in which the intensities are within a range of 25% - 75% of the data), the upper and lower quantiles (dashed lines, spectra in which the intensities are within a range of 1% - 99%) as well as the outliers (spectra with intensities greater than 99% and lower than 1% of the data).

**Figure S6.** MALDI imaging mass spectrometry (IMS) shows production of 88 m/z ions in the *P. fluorescens* SS101-*N. americana* (A) and *P. fluorescens* SBW25-*N. americana* (B) interactions respectively. a.u. = arbitrary units.

**Figure S7.** (A) MALDI imaging mass spectrometry (IMS) shows production of 311-477 m/z ions and its cluster ions in the MS/MS network in *P. fluorescens* SBW25-*N. americana* interaction. a.u. = arbitrary units. (B) MS/MS profile of 325 m/z during the *P. fluorescens* SBW25-*N. americana*. (C) MALDI imaging mass spectrometry (IMS) shows production of 752-809 m/z ions and its cluster ions in the MS/MS network in *P. fluorescens* SBW25-*N. americana* interaction. a.u. = arbitrary units. (D) MS/MS profile of 766 m/z during the *P. fluorescens* SBW25-*N. americana*

**Figure S8.** MS/MS profile of 843 m/z (A) and 868 m/z (B) during the *P. fluorescens* SBW25- *N. americana* interaction.

**Figure S9.** MS/MS profile of 883 m/z (A) and 897 m/z (B) during the *P. fluorescens* SBW25-*N. americana* interaction.

**Table S1.** Whole genome transcriptome analysis of *P. fluorescens* strain SS101 in the presence of *N. americana*

**Table S2.** Co-localization ions of the MALDI-IMS of *P. fluorescens* strain SS101 in the presence of *N. americana*

**Table S3.** Whole genome transcriptome analysis of *P. fluorescens* strain SBW25 in the presence of *N. americana*

**Table S4.** Ion clusters of the lipopeptide massetolide A and its derivatives in *P. fluorescens* strain SS101 interaction with *N. americana*

**Table S5.** Ion cluster of 325-477 m/z in *P. fluorescens* strain SS101 interaction with *N. americana*

**Table S6.** Ion cluster of 766-796 m/z in *P. fluorescens* strain SS101 interaction with *N. americana*

Figure S1

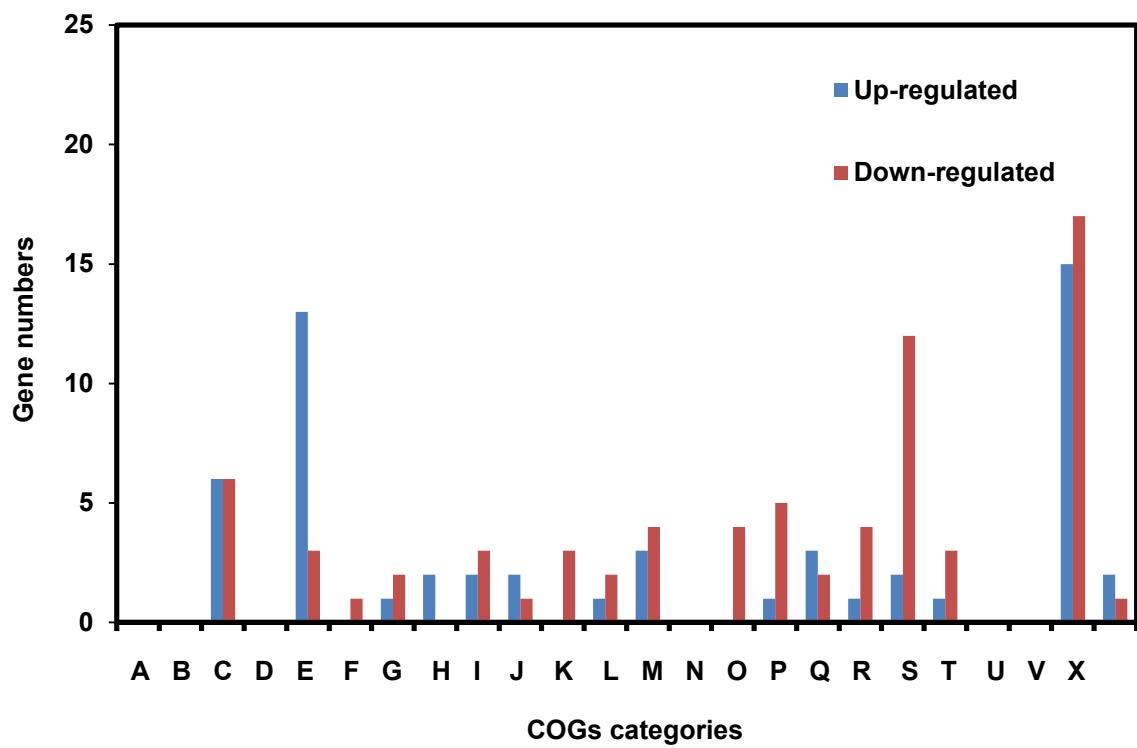


Figure S2

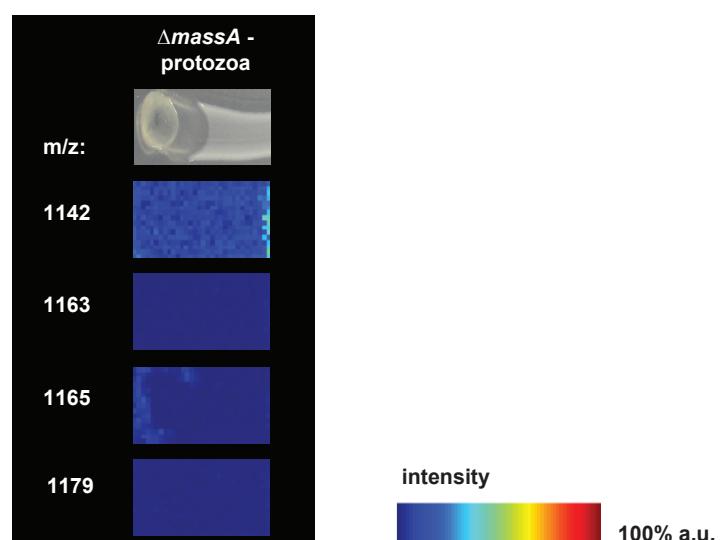


Figure S3

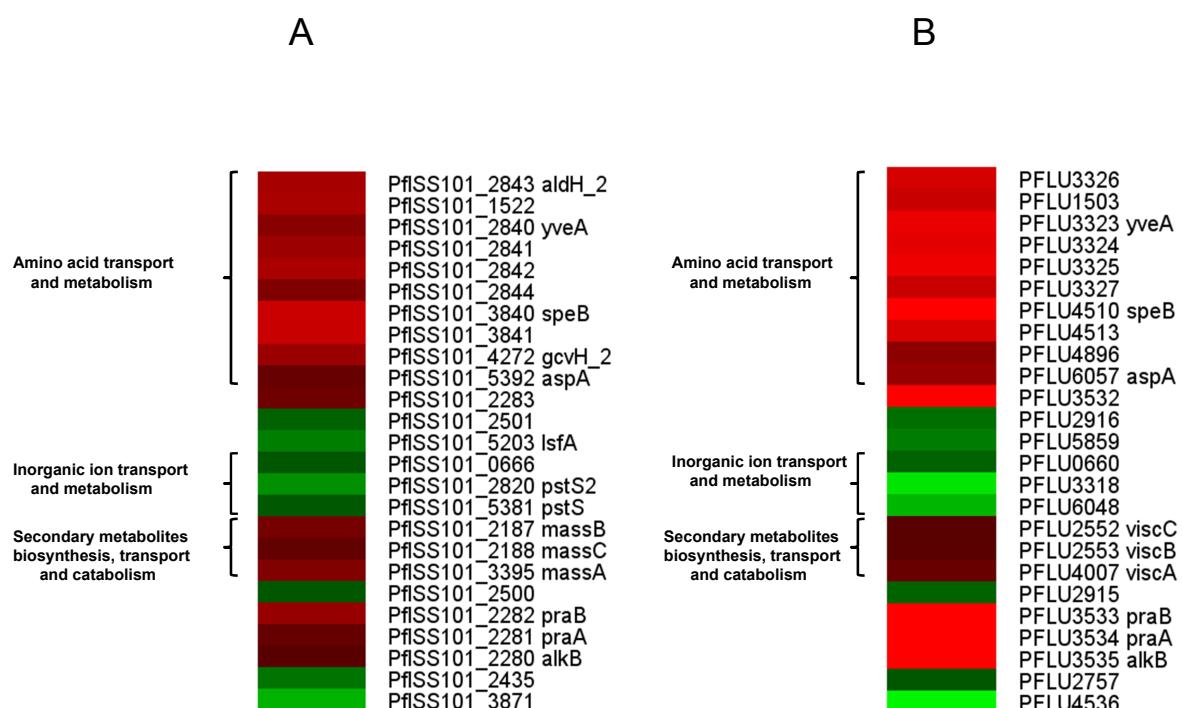
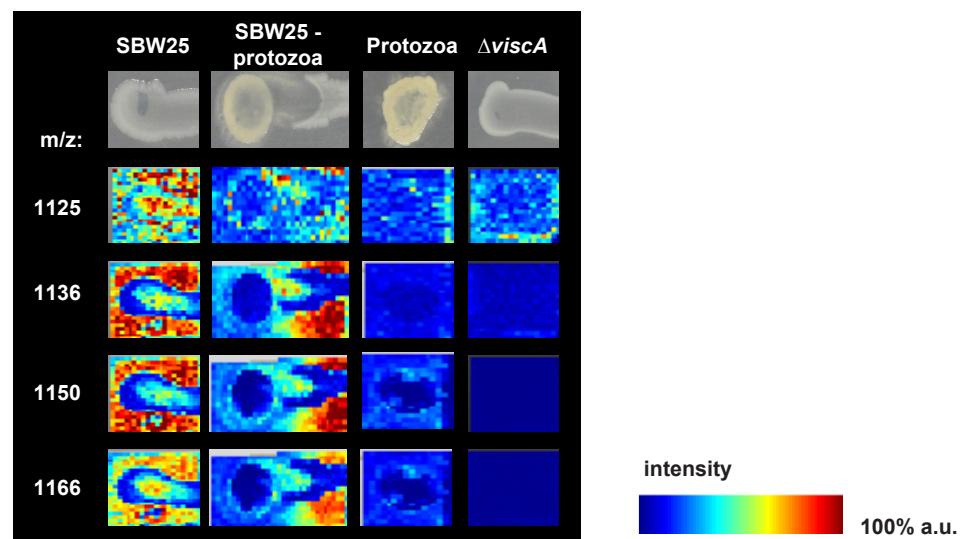
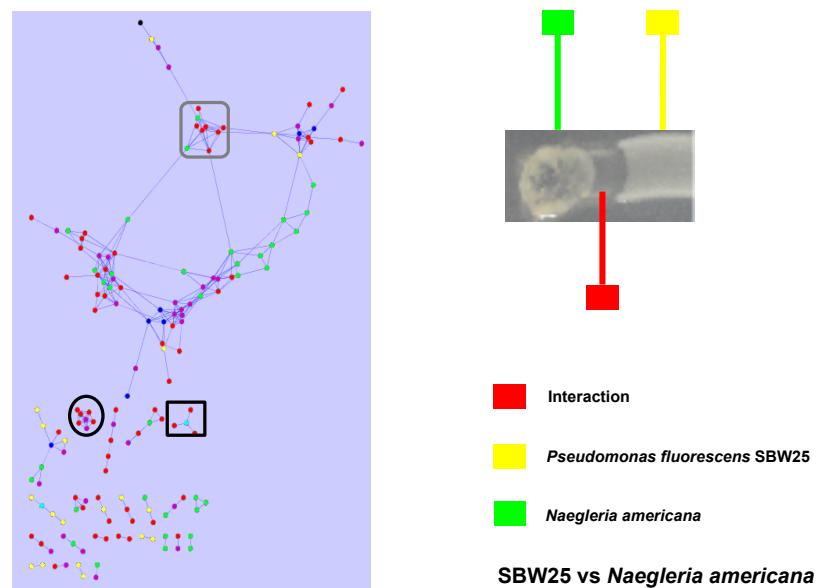


Figure S4

A



B



C

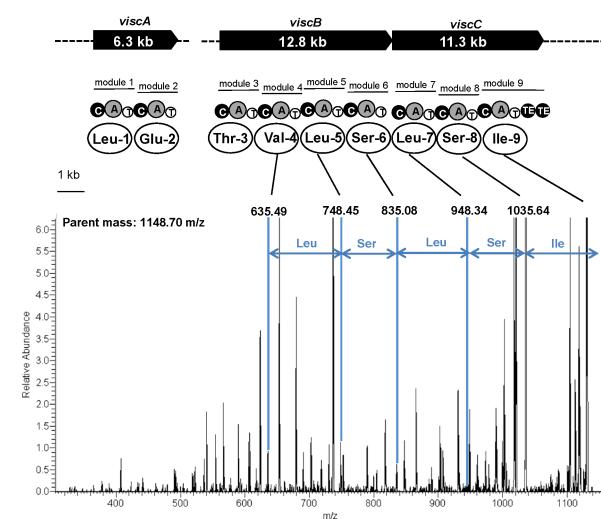


Figure S5

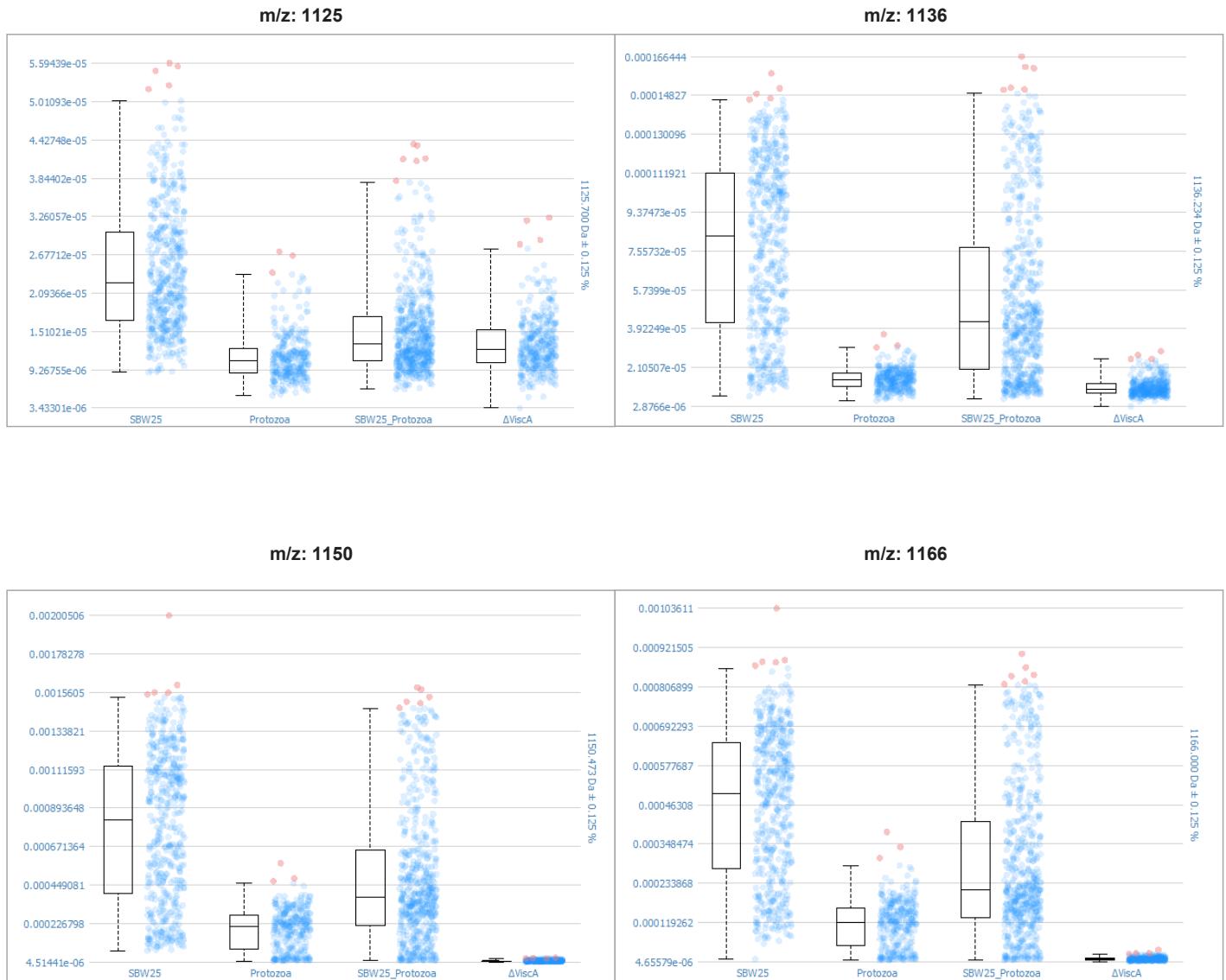


Figure S6

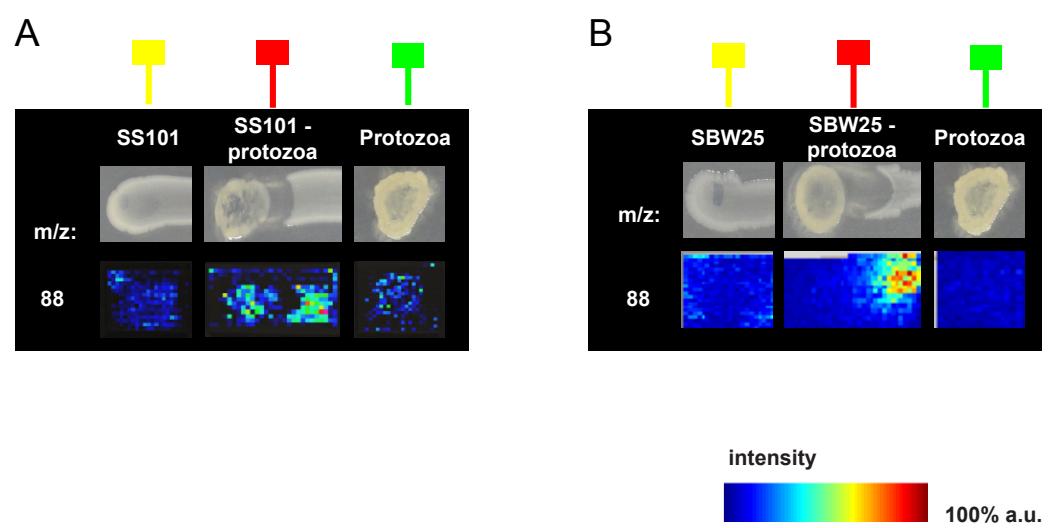


Figure S7

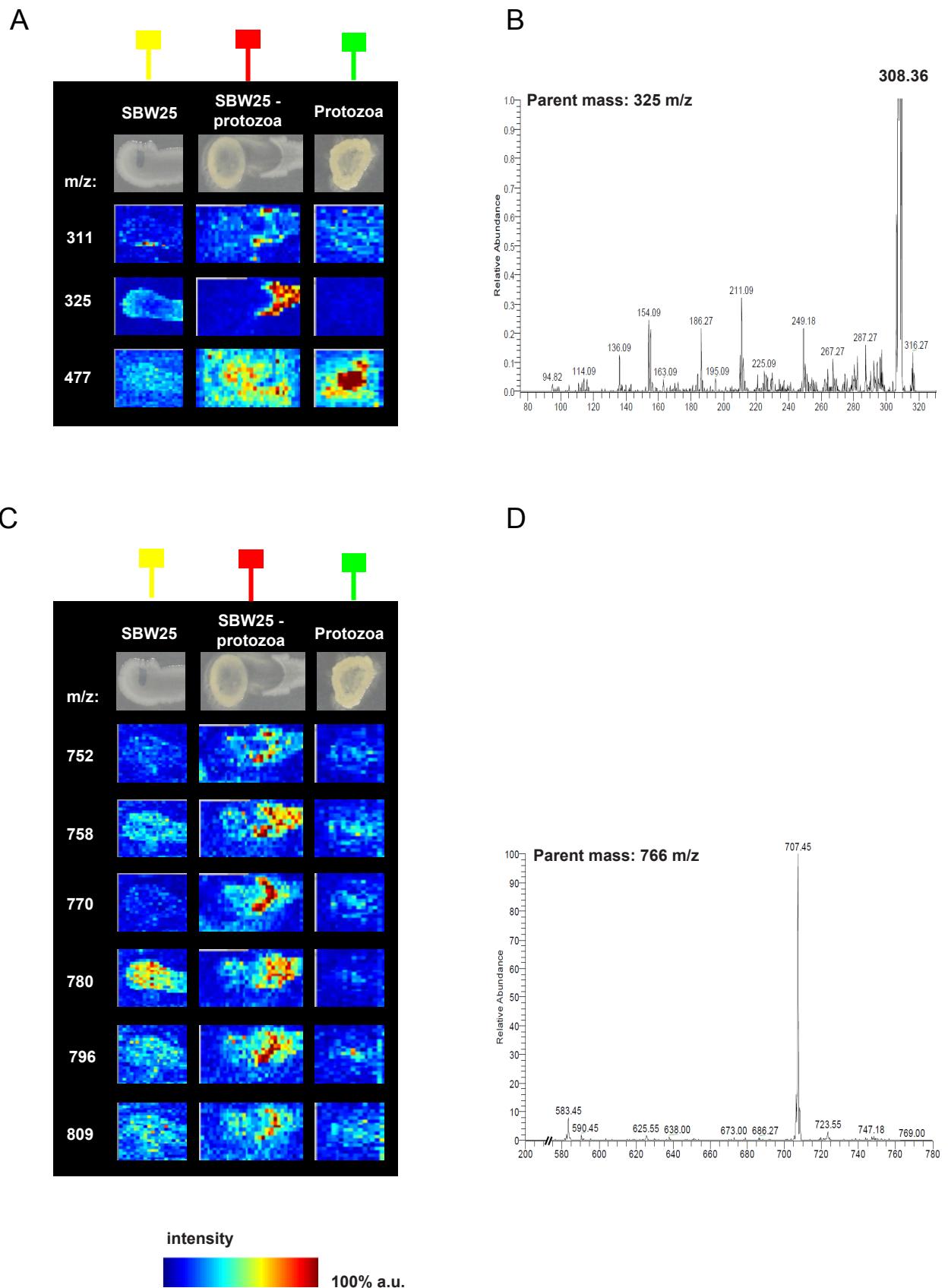
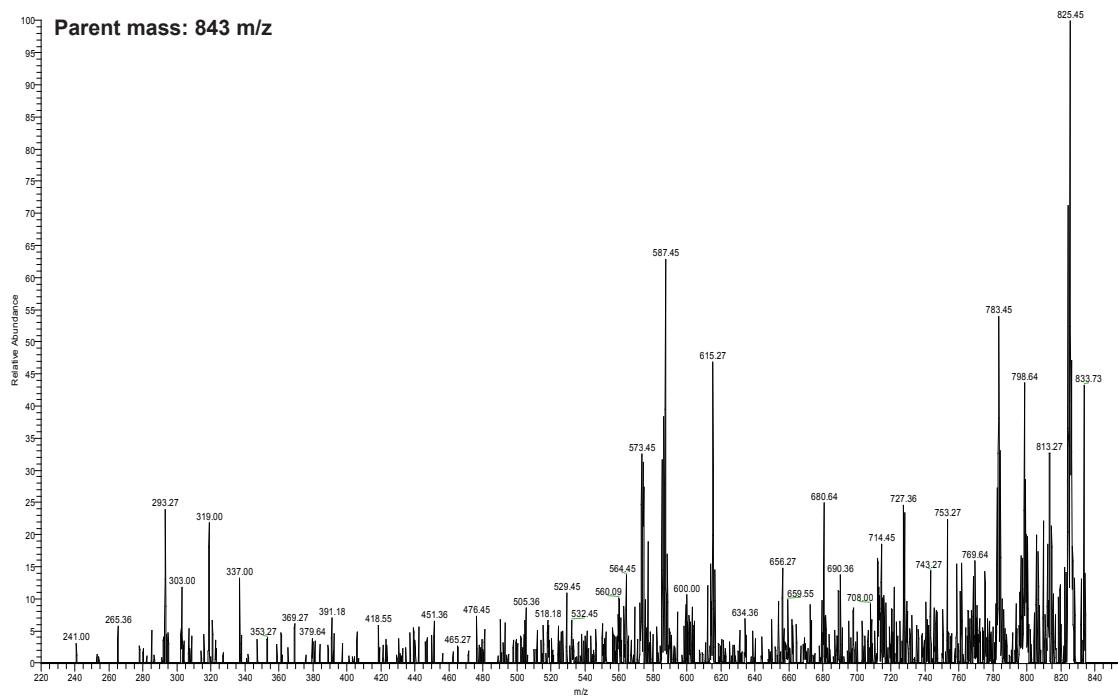


Figure S8

A



B

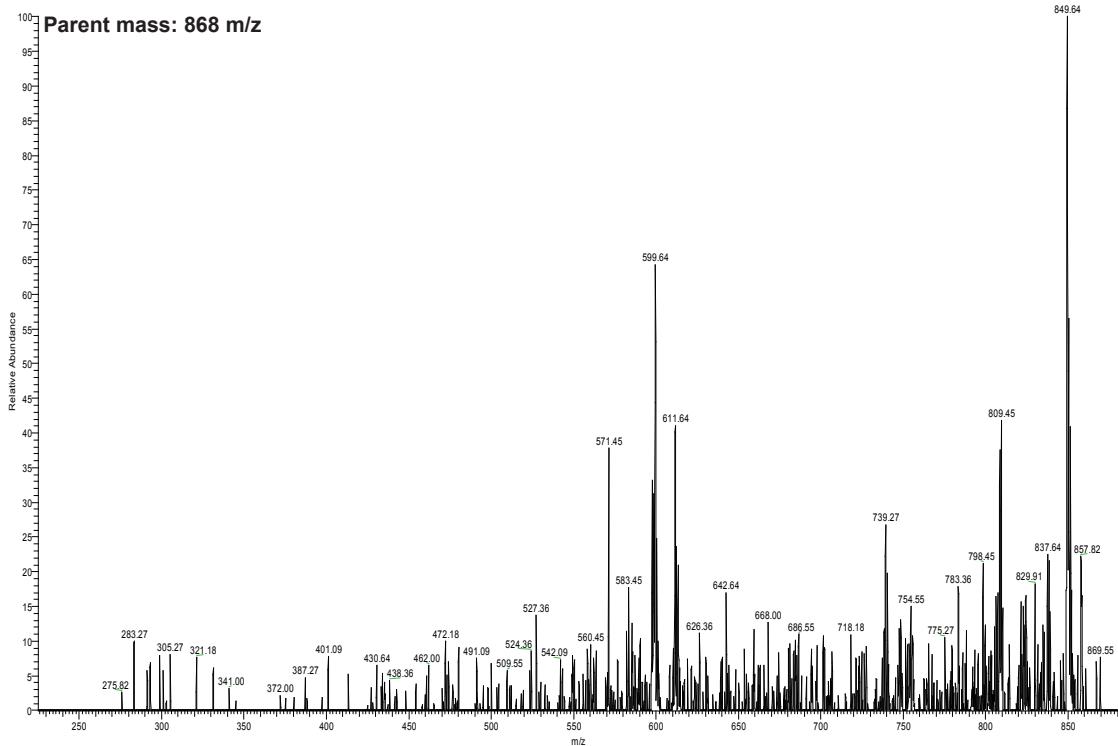
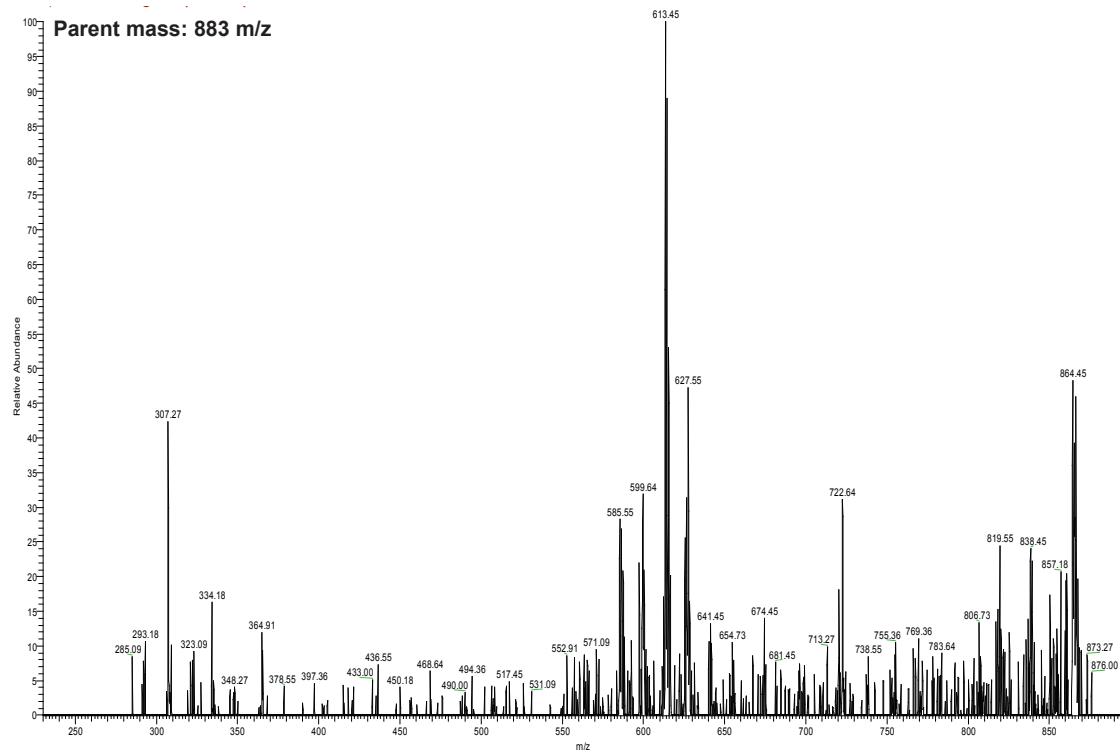
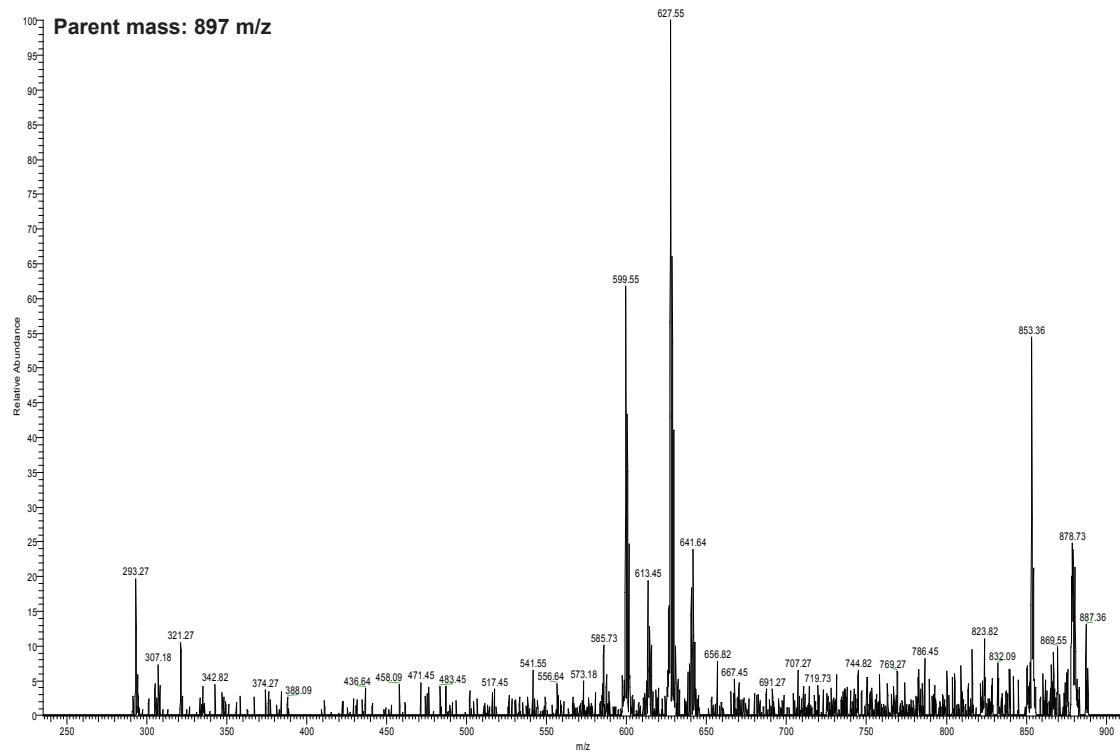


Figure S9

A



B





Light blue class			
m/z at peak centroid	± m/z interval	m/z with maximal correlation value	Maximal correlation value
994.215	1.243	994.18	0.507
1136.038	1.42	1136.063	0.672
1142.68	1.428	1142.667	0.579
1150.44	1.779	1151.032	0.727
1165.377	1.457	1165.796	0.606
1181.805	1.477	1181.856	0.539
Green class			
m/z at peak centroid	± m/z interval	m/z with maximal correlation value	Maximal correlation value
249.575	0.312	249.575	0.559
265.578	0.332	265.548	0.588
445.112	0.556	445.12	0.543
461.112	0.576	461.136	0.595
476.833	0.335	477.16	0.518
477.503	0.335	477.225	0.54
672.38	0.84	672.361	0.588
688.539	0.861	688.519	0.511
740.469	0.473	740.922	0.517
741.415	0.473	740.962	0.504
766.87	0.959	766.87	0.549
1163.397	1.454	1163.003	0.676
1179.645	1.475	1179.121	0.699
1201.933	1.502	1201.43	0.661
Orange class			
m/z at peak centroid	± m/z interval	m/z with maximal correlation value	Maximal correlation value
183.895	0.23	183.95	0.512
223.928	0.28	223.917	0.568
257.939	0.173	258.089	0.502
258.286	0.173	258.136	0.504
Dark red class			
m/z at peak centroid	± m/z interval	m/z with maximal correlation value	Maximal correlation value
43.338	0.229	43.25	0.687
43.611	0.045	43.567	0.549
88.292	0.064	88.339	0.519
88.42	0.064	88.374	0.502
194.579	0.243	194.579	0.568
232.825	0.291	232.774	0.76
281.931	0.352	281.956	0.529
284.791	0.195	284.951	0.506
285.181	0.195	285.039	0.526
313.524	0.768	313.136	0.857
325.504	0.676	325.27	0.828
326.495	0.314	326.207	0.585
328.747	0.221	328.944	0.523
329	0.032	329.012	0.513
329.254	0.223	329.039	0.525
353.334	0.442	353.251	0.623
369.37	0.462	369.249	0.776
373.272	0.467	373.179	0.539
454.097	0.292	454.373	0.512
454.68	0.292	454.404	0.53
552.543	1.152	551.949	0.871
555.688	0.695	555.705	0.516
564.888	1.562	564.12	0.896
576.032	0.72	575.96	0.753
592.353	0.876	592.217	0.847
604.505	1.013	604.13	0.877
616.27	0.491	616.549	0.572
616.955	0.194	617.121	0.561
617.622	0.474	617.213	0.565
626.435	0.836	626.193	0.839
648.201	0.81	648.219	0.77
684.044	0.855	684.063	0.605
703.918	0.46	704.339	0.507
704.58	0.202	704.713	0.637
705.406	0.623	704.812	0.639
715.294	1.162	714.767	0.88
727.495	1.162	727.535	0.885
737.188	0.642	737.811	0.571
738.199	0.368	738.416	0.678
739.216	0.649	738.618	0.682
743.482	0.929	743.675	0.847
747.093	0.934	747.164	0.664
750.631	1.293	750.498	0.769
765.276	0.957	765.307	0.683
769.257	0.507	769.753	0.52
770.271	0.507	769.774	0.514
780.708	0.976	780.677	0.52
790.017	0.988	789.985	0.511
957.25	1.197	957.296	0.569



Parent mass	Classes
970.591	T
1140.726	T
1148.68	T
1157.742	T
1158.77	T
1162.681	S
1162.709	ST
1163.767	ST
1164.85	T
1178.684	ST
1178.687	S
1180.69	T
1182.67	T
1184.77	T
1186.95	T

Parent mass	Classes
311.307	protozoa and interaction
325.322	Interaction
325.323	Interaction
451.461	protozoa and interaction
451.462	Interaction
477.478	Interaction
477.478	Interaction
477.478	Interaction
477.479	Interaction

Parent mass	Classes
752.518	T
766.536	T
768.495	T
770.517	S
772.594	T
782.513	T
782.514	T
782.56	T
796.531	AT