

## Supplementary Information

Facilitating imaging mass spectrometry of microbial specialized metabolites with METASPACE.

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This file contains Supplementary Tables 1-4 and Supplementary Figures 1-2.

Supplementary Table S1. Bacterial strains used for evaluation of microbial imaging mass spectrometry (imaging MS) with METASPACE.

Class	Genus and species	Strain Number
Actinobacteria	<i>Rhodococcus erythropolis</i>	43066
Actinobacteria	<i>Rhodococcus opacus</i>	43205
Actinobacteria	<i>Streptomyces coelicolor</i>	A2(3) / M145
Actinobacteria	<i>Streptomyces griseus subsp griseus</i>	40236
Actinobacteria	<i>Streptomyces mobaraensis</i>	
Actinobacteria	<i>Streptomyces lividans</i>	PM66
Actinobacteria	<i>Streptomyces roseolilacinus</i>	40173
Gamma Proteobacteria	<i>Pseudomonas savastanoi</i>	722
Gamma Proteobacteria	<i>Pseudomonas putida</i>	RW10S2
Bacilli	<i>Bacillus subtilis BGA</i>	618

Supplementary Table S2. Number of metabolites and unique molecular formulas from the Natural Products Atlas (NPA) compared to the number METASPACE annotations at various FDR percentages, with and without off-sample filtering.

Metabolites in NPA	Unique molecular formulas in NPA	Total number of METASPACE annotations using NPA (FDR)							
		No filtering				Off-sample filtering			
		50%	20%	10%	5%	50%	20%	10%	5%
5018	3727	1968	95	29	18	1045	64	20	12

Supplementary Table S3. Number of metabolites and unique molecular formulas from NPA after filtering for bacterial genus and species as compared to the number of unique molecular formulas annotated by METASPACE. Unique molecular formulas annotated by METASPACE are broken down by FDR 50% vs 20% and whether off-sample filtering was used.

Genus	species	Metabolites in NPA	Unique molecular formulas in NPA	Unique molecular formulas annotated by METASPACE				Curated filtering
				FDR 50% no filter	FDR 50% off-sample filter	FDR 20% no filter	FDR 20% off-sample filter	
<i>Bacillus</i>	–	389	275	40	26	2	2	9
	<i>subtilis</i>	101	81	8	5	2	2	9
<i>Pseudomonas</i>	–	266	235	39	27	2	1	10
	<i>putida</i>	3	2	0	0	0	0	9
	<i>savastanoi</i>	2	2	0	0	0	0	1
<i>Rhodococcus</i>	–	20	16	4	1	0	0	0
	<i>erythropolis</i>	3	3	1	0	0	0	0
	<i>opacus</i>	0	0	0	0	0	0	0
<i>Streptomyces</i>	–	4343	3201	512	285	30	22	34
	<i>coelicolor</i>	25	23	8	7	1	1	3
	<i>griseus</i>	106	89	24	15	0	0	3
	<i>lividans</i>	9	9	2	1	0	0	1
	<i>mobaraensis</i>	1	1	0	0	0	0	27
	<i>roseolilacinus</i>	0	0	0	0	0	0	0

Supplementary Table S4. 53 curated annotations from Figure 2 and Supplementary Figure 1 with direct URL to the annotation in METASPACE. Ordering is based on decreasing MSM score.

FDR	MSM	Formula	Adduct	m/z	Molecule Names
5%	0.974	C54H95N9O16	+Na	1148.679	['WLIP; Viscosin; Massetolide F', 'Massetolide F', 'Viscosin'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C54H95N9O16&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C54H95N9O16&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
5%	0.927	C25H37NO4	+H	416.2795	['Trichobotrysin C', 'Piericidin A1', 'Phomacin C', 'Trichobotrysin B'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H37NO4&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H37NO4&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
5%	0.902	C51H80N12O14	+Na	1107.581	['Iturin A-8', 'Mycosubtilin D', 'Iturin A9'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C51H80N12O14&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C51H80N12O14&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
5%	0.872	C54H95N9O16	+K	1164.653	['WLIP; Viscosin; Massetolide F', 'Massetolide F', 'Viscosin'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C54H95N9O16&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C54H95N9O16&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
5%	0.848	C51H80N12O14	+K	1123.555	['Iturin A-8', 'Mycosubtilin D', 'Iturin A9'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C51H80N12O14&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C51H80N12O14&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
20%	0.777	C25H37NO4	+Na	438.2614	['Trichobotrysin C', 'Piericidin A1', 'Phomacin C', 'Trichobotrysin B'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H37NO4&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H37NO4&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
20%	0.733	C53H93N7O13	+Na	1058.672	['[Ile7]-surfactin', 'Pumilacidin B', 'Leu7-Surfactin', 'Not named', 'Not named', 'Not named', 'Gageopeptin A'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C53H93N7O13&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C53H93N7O13&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.729	C53H93N9O16	+K	1150.637	['Massetolide E', 'Massetolide E'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C53H93N9O16&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C53H93N9O16&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.717	C25H37NO4	+K	454.2354	['Trichobotrysin C', 'Piericidin A1', 'Phomacin C', 'Trichobotrysin B'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H37NO4&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H37NO4&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.619	C53H93N7O13	+K	1074.646	['[Ile7]-surfactin', 'Pumilacidin B', 'Leu7-Surfactin', 'Not named', 'Not named', 'Not named', 'Gageopeptin A'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C53H93N7O13&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C53H93N7O13&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.611	C25H33N3O	+H	392.2696	['Butylcyclohexylprodigiosin', 'Metacycloprodigiosin', 'Methyl-cyclodecylprodiginine', 'Propyl-metacyclooctylprodiginine'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H33N3O&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H33N3O&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.594	C25H35N3O	+H	394.2852	['Undecylprodigiosin', 'Prodigiosin 25-C'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H35N3O&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H35N3O&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>



Supplementary Table S4 continued.

<b>FDR</b>	<b>MSM</b>	<b>Formula</b>	<b>Adduct</b>	<b>m/z</b>	<b>Molecule Names</b>
50%	0.541	C <sub>14</sub> H <sub>19</sub> NO <sub>4</sub>	+H	266.1386	['3097-B2', 'Salinosporamide D', 'Fuzanin B', 'Fuzanin H', 'Salinosporamide G', 'Fuzanin A', 'Anisomycin'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C14H19NO4&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C14H19NO4&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
20%	0.459	C <sub>53</sub> H <sub>93</sub> N <sub>9</sub> O <sub>16</sub>	+Na	1134.663	['Massetolide E', 'Massetolide E'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C53H93N9O16&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C53H93N9O16&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.438	C <sub>28</sub> H <sub>36</sub> N <sub>4</sub> O <sub>6</sub>	+Na	547.2527	['Safracin A'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C28H36N4O6&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C28H36N4O6&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.277	C <sub>14</sub> H <sub>19</sub> NO <sub>4</sub>	+Na	288.1206	['3097-B2', 'Salinosporamide D', 'Fuzanin B', 'Fuzanin H', 'Salinosporamide G', 'Fuzanin A', 'Anisomycin'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C14H19NO4&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C14H19NO4&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.188	C <sub>14</sub> H <sub>19</sub> NO <sub>4</sub>	+K	304.0945	['3097-B2', 'Salinosporamide D', 'Fuzanin B', 'Fuzanin H', 'Salinosporamide G', 'Fuzanin A', 'Anisomycin'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C14H19NO4&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C14H19NO4&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.185	C <sub>18</sub> H <sub>36</sub> N <sub>6</sub> O <sub>5</sub>	+H	417.282	['2"-N-formimidoylistamycin B"] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C18H36N6O5&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C18H36N6O5&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.169	C <sub>54</sub> H <sub>95</sub> N <sub>9</sub> O <sub>16</sub>	+H	1126.697	['WLIP; Viscosin; Massetolide F', 'Massetolide F', 'Viscosin'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C54H95N9O16&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C54H95N9O16&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.14	C <sub>56</sub> H <sub>99</sub> N <sub>9</sub> O <sub>16</sub>	+Na	1176.71	['Massetolide B/H', 'Massetolide H', 'Massetolide B'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C56H99N9O16&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C56H99N9O16&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.135	C <sub>25</sub> H <sub>37</sub> NO <sub>3</sub>	+Na	422.2665	['Trichobotrysin D', 'Piericidin F'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H37NO3&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H37NO3&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.126	C <sub>25</sub> H <sub>37</sub> NO <sub>3</sub>	+H	400.2846	['Trichobotrysin D', 'Piericidin F'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H37NO3&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H37NO3&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.125	C <sub>34</sub> H <sub>46</sub> N <sub>2</sub> O <sub>8</sub>	+K	649.2885	['UCF-116-C', 'Ansatrienin A3', 'Ansatrienin A2'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C34H46N2O8&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C34H46N2O8&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.124	C <sub>20</sub> H <sub>27</sub> NO <sub>4</sub>	+H	346.2012	['Radiospene J', '(2E)-11-(4'-aminophenyl)-5,9-O-cyclo-4,6,8-trimethyl-11-oxo-undec-2-enoic acid"] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C20H27NO4&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C20H27NO4&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>

Supplementary Table S4 continued.

<b>FDR</b>	<b>MSM</b>	<b>Formula</b>	<b>Adduct</b>	<b>m/z</b>	<b>Molecule Names</b>
50%	0.122	C52H91N7O13	+Na	1044.657	['Not named', '[Ile4]Surfactin', '[Leu4]Surfactin', 'Not named', '[Leu-7]surfactin', '[Ile2,4,7]Surfactin', 'Not named', 'Surfactin', 'Gageopeptin B']
<a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C52H91N7O13&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C52H91N7O13&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>					
50%	0.121	C56H99N9O16	+K	1192.684	['Massetolide B/H', 'Massetolide H', 'Massetolide B']
<a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C56H99N9O16&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C56H99N9O16&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>					
50%	0.119	C10H13NO4	+H	212.0917	['Farinomalein', 'N-salicyloyl-2-aminopropan-1,3-diol', 'Pestalactam B', '2,5-Dihydroxy-4-methoxymethyl-acetanilide', 'Makomotine D', 'CJ-14,877']
<a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C10H13NO4&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C10H13NO4&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>					
50%	0.118	C40H70O8	+K	717.4702	['Campechic acid A']
<a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C40H70O8&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C40H70O8&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>					
50%	0.118	C55H97N9O16	+Na	1162.695	['Pseudophomin A; Massetolide A/D/G', 'Massetolide A', 'Massetolide D', 'Massetolide G', 'Pseudophomin A']
<a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C55H97N9O16&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C55H97N9O16&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>					
50%	0.113	C27H35NO4	+H	438.2638	['Heronamide D']
<a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C27H35NO4&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C27H35NO4&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>					
50%	0.111	C55H97N9O16	+K	1178.668	['Pseudophomin A; Massetolide A/D/G', 'Massetolide A', 'Massetolide D', 'Massetolide G', 'Pseudophomin A']
<a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C55H97N9O16&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C55H97N9O16&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>					
50%	0.106	C24H35NO3	+Na	408.2509	['Ascosalipyrrolidinone B', 'TMC-169', 'Mer-A2026B']
<a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C24H35NO3&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C24H35NO3&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>					
50%	0.097	C50H78N12O14	+Na	1093.565	['Iturin A-7', 'Iturin A6']
<a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C50H78N12O14&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C50H78N12O14&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>					
50%	0.093	C13H16N2O3	+H	249.1233	['Salinazinone B', 'Abbeymycin', 'Spinnoxazine B']
<a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C13H16N2O3&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C13H16N2O3&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>					
50%	0.089	C24H35NO3	+H	386.2689	['Ascosalipyrrolidinone B', 'TMC-169', 'Mer-A2026B']
<a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C24H35NO3&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C24H35NO3&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>					
50%	0.087	C43H51NO16	+K	876.2839	['Sulfurmycin B']
<a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C43H51NO16&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C43H51NO16&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>					

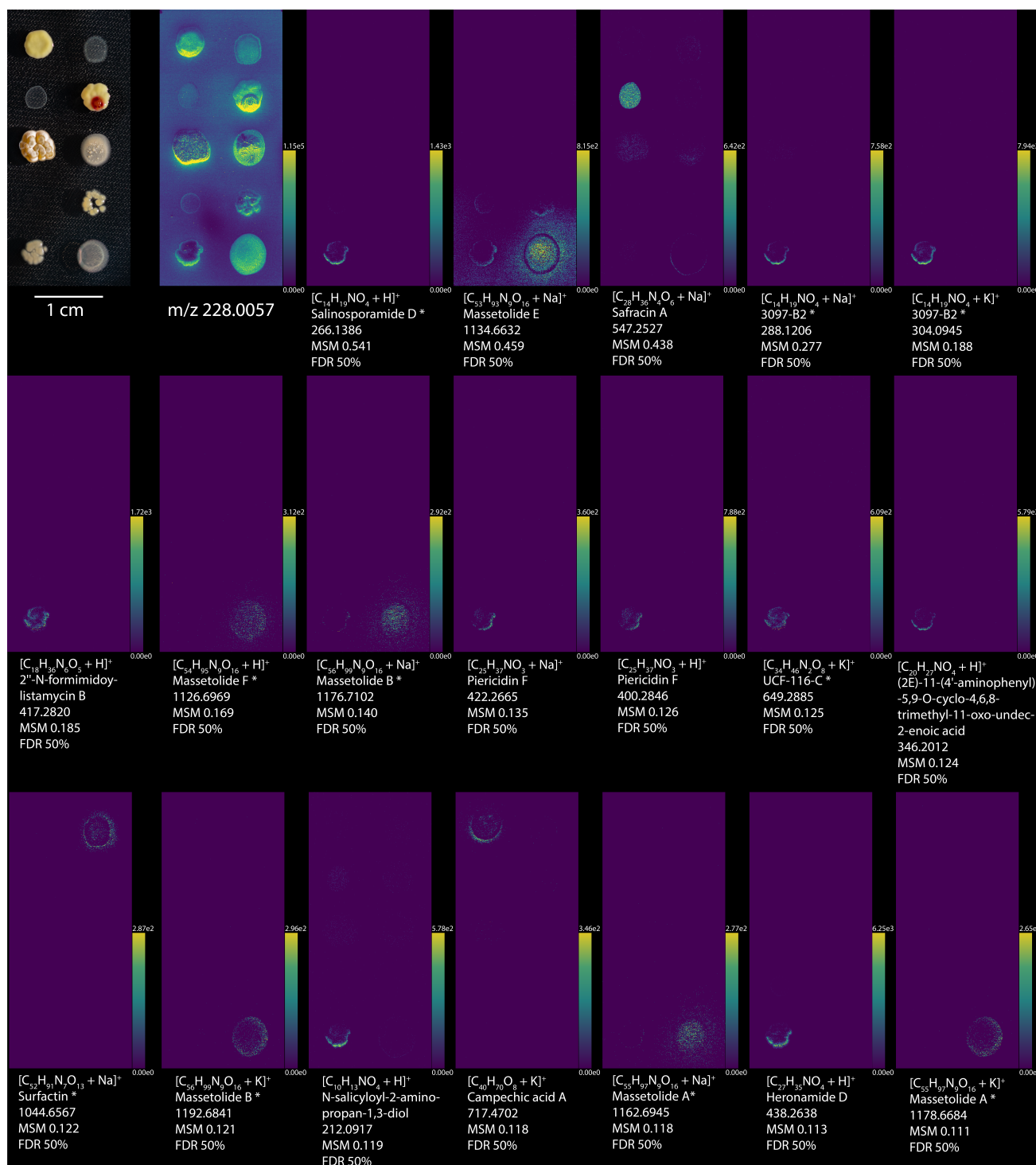
Supplementary Table S4 continued.

<b>FDR</b>	<b>MSM</b>	<b>Formula</b>	<b>Adduct</b>	<b>m/z</b>	<b>Molecule Names</b>
50%	0.079	C24H35NO4	+Na	424.2458	['Aspochalasin J', 'SN-I98-D', 'L-755807', 'Gymnastatin H', 'Aspergillin PZ', 'Aspochalasin P', 'Aspochalasin D', 'Trichalasin H', 'Aspochalasin M', 'Aspochalasin S', 'Piericidin E1'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C24H35NO4&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C24H35NO4&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.079	C24H35NO3	+K	424.2248	['Ascosalipyrrolidinone B', 'TMC-169', 'Mer-A2026B'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C24H35NO3&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C24H35NO3&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.071	C23H33NO5	+H	404.2431	['Salternamide B', 'Neopestalotin C', '12'-hydroxy gymnastatin N', 'Chartarutine A'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C23H33NO5&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C23H33NO5&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.064	C23H33NO5	+Na	426.2251	['Salternamide B', 'Neopestalotin C', '12'-hydroxy gymnastatin N', 'Chartarutine A'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C23H33NO5&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C23H33NO5&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.063	C20H29NO5	+H	364.2118	['(2E)-11-(4-aminophenyl)-5,9-dihydroxy-4,6,8-trimethyl-11-oxo-undec-2-enoic acid'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C20H29NO5&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C20H29NO5&amp;add=%2BH&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.062	C18H36N6O5	+K	455.2378	['2"-N-formimidoylistamycin B'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C18H36N6O5&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C18H36N6O5&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.059	C26H39NO3	+Na	436.2822	['Termitomycamide A', 'Trichobotrysin E', 'Myceliothermophin B', 'Myceliothermophin A', 'Mer-A2026A'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C26H39NO3&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C26H39NO3&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.057	C29H54N10O12	+Na	757.3814	['Tsukubachelin B'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C29H54N10O12&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C29H54N10O12&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.057	C50H77N11O15	+Na	1094.549	['Iturin C3'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C50H77N11O15&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C50H77N11O15&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.055	C25H37NO3	+K	438.2405	['Trichobotrysin D', 'Piericidin F'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H37NO3&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C25H37NO3&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.036	C50H78N12O14	+K	1109.539	['Iturin A-7', 'Iturin A6'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C50H78N12O14&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C50H78N12O14&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.028	C52H91N7O13	+K	1060.631	['Not named', '[Ile4]Surfactin', '[Leu4]Surfactin', 'Not named', '[Leu-7]surfactin', '[Ile2,4,7]Surfactin', 'Not named', 'Surfactin', 'Gageopeptin B'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C52H91N7O13&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C52H91N7O13&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>

Supplementary Table S4 continued.

<b>FDR</b>	<b>MSM</b>	<b>Formula</b>	<b>Adduct</b>	<b>m/z</b>	<b>Molecule Names</b>
50%	0.025	C47H78O14	+Na	889.5283	['Endusamycin'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C47H78O14&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C47H78O14&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.022	C23H42O7	+K	469.2562	['8-Deoxylankolide'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C23H42O7&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C23H42O7&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.02	C20H27NO4	+Na	368.1832	['Radiaspene J', "(2E)-11-(4'-aminophenyl)-5,9-O-cyclo-4,6,8-trimethyl-11-oxo-undec-2-enoic acid"] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C20H27NO4&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C20H27NO4&amp;add=%2BNa&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.013	C17H29N7O8	+K	498.1709	['Glycinothricin'] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C17H29N7O8&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C17H29N7O8&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>
50%	0.013	C20H27NO4	+K	384.1571	['Radiaspene J', "(2E)-11-(4'-aminophenyl)-5,9-O-cyclo-4,6,8-trimethyl-11-oxo-undec-2-enoic acid"] <a href="https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C20H27NO4&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula">https://metaspace2020.eu/annotations?db=NPA-2019-08&amp;ds=2020-01-07_11h15m21s&amp;mol=C20H27NO4&amp;add=%2BK&amp;fdr=0.5&amp;sections=3&amp;sort=formula</a>

Supplementary Figure S1. Imaging MS and annotated molecules by METASPACE using NPA. All annotations filtered by organism type, genus, and then hand selected for reasonable ion distribution, resulting in the 13th to 53rd highest ranked an-notations. Optical image of *Streptomyces griseus* subsp *griseus* 40236, *Bacillus subtilis* BGA 618, *Pseudomonas savastanoi* 722, *Streptomyces coelicolor* A2(3) / M145, *Streptomyces lividans* PM66, *Rhodococcus erythropolis* 43066, *Rhodococcus opacus* 43205, *Streptomyces roseililacinus* 40173, *Streptomyces mobaraensis*, *Pseudomonas putida* RW10S2, from left to right, top to bottom. Ion images are arranged from increasing to decreasing MSM score from left to right, top to bottom, while  $m/z$  228 was selected as a background ion image to show the locations of all the bacteria. Each ion image is denoted by the adduct detected, molecule name, the monoisotopic  $m/z$  value, MSM score, and FDR percentage. Molecule names with an \* represent annotations with potential analogs.





Supplementary Figure S2. Average mass spectrum of the entire imaging area (outlined in pink) including all 10 microbes. The imzML file was loaded into MSiReader (v1.01) and the polygon tool was used to select the region of interest, followed by exporting and viewing the average mass spectrum from the selected pixels.

