

Near real-time analysis of the phenotypic responses of *Escherichia coli* to 1-butanol exposure using Raman spectroscopy

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Supplementary Appendix

Supplementary Table 1. Correlation coefficients (R values) for FAME analysis data with Raman spectroscopy data using Raman bands assigned in the literature and new bands identified in this research.

Raman Band (cm ⁻¹)	Saturated Fatty Acids (R)	Unsaturated Fatty Acids (R)	Cyclopropane Fatty Acids (R)	Raman Signature Assignment	References
877	0.014	-	-	Symmetric stretching (C-C-N ⁺)	(1)
968	-	-	-	Lipids	(1)
980	0.20	-	-	Bending (=CH)	(1)
1057	-	0.37	-	Lipids	(1)
1064	-	0.09	-	Vibrational stretch (C-C)	(1)
1078	-	-	-	Vibrational stretch (C-C) (C-O)	(1)
1095	-	0.64	-	Lipids	(1)
1123	0.33	-	0.56	Vibrational stretch (C-C)	(1, 2)
1131	0.02	-	0.37	Fatty Acid	(1)
1168	-	-	0.14	Lipid Assignment (C=C) (COH)	(1)
1249	-	0.53	-	Bend (=CH)	(3)
1250	-	0.57	-	Bend (=CH)	(3)
1251	-	0.60	-	Bend (=CH)	(3)
1252	-	0.59	-	Bend (=CH)	(3)
1253	-	0.60	-	Bend (=CH)	(3)
1254	-	0.60	-	Bend (=CH)	(3)
1255	-	0.61	-	Lipids, Bend (=CH)	(1, 3)
1256	-	0.62	-	Bend (=CH)	(3)
1257	--	0.64	-	Bend (=CH)	(3)

1258	-	0.66	-	Bend (=CH)	(3)
1259	-	0.69	-	Bend (=CH)	(3)
1260	-	0.72	-	Bend, <i>cis</i> Stretch (=CH), Deformation (-CH ₂)	(1, 3, 4)
1263	-	0.76	-	Bend, Symmetric Rocking (=CH)	(2, 3)
1264	-	0.75	-	Bend (=CH)	(3)
1265	-	0.71	-	Bend (=CH)	(3)
1266	-	0.64	-	Bend (=CH), <i>cis</i> Deformation (=CH)	(1, 3, 5)
1267	-	0.55	-	Lipid (CH), Bend (=CH)	(1, 3)
1268	-	0.46	-	Deformation (=CH)	(1)
1270	-	0.40	-	Lipid assignment (C=C)	(1)
1298	-	0.12	0.30	Palmitic Acid, Acyl Chains, Fatty Acids	(1)
1299	-	0.03	0.40	Deformation (CH ₂)	(1)
1300	0.07	-	0.48	Twist (-CH ₂)	(2)
1301	0.16	-	0.54	Vibration (-CH) Twist (-CH ₂)	(1, 3)
1302	0.23	-	0.59	Lipid Assignment, Bend, Twist (-CH ₃) (-CH ₂)	(1)
1304	0.29	-	0.64	Deformation (-CH ₂)	(1)
1307	0.2	-	0.60	Bend, Twist (-CH ₃) (-CH ₂)	(1)
1309	0.02	-	0.49	Bend, Twist (-CH ₃) (-CH ₂)	(1)
1313	-	0.31	0.11	Twist (-CH ₂ CH ₃)	(1)
1367	-	0.03	0.47	Phospholipids, Symmetric Vibration (-CH ₃)	(1)
1379	-	0.02	0.31	Lipid Assignment	(1)
1393	0.16	-	0.53	Rocking (CH)	(1)
1437	0.10	-	-	Deformation (CH ₂)	(1)
1439	0.2	-	-	Deformation, Scissoring (CH ₂)	(1)
1440	0.24	-	-	Deformation (CH) (CH ₂) (CH ₃)	(1)

1441	0.28	-	-	Deformation, Bend, Scissoring (CH) (CH ₂) (CH ₃)	(1)
1442	0.31	-	-	Fatty Acids, Deformation, Bend (CH ₂) (CH ₃)	(1)
1443	0.32	-	-	Fatty Acids, Deformation (CH ₂)	(1)
1444	0.32	-	-	Fatty Acids, Deformation (CH ₂)	(1)
1445	0.3	-	-	Phospholipids, Deformation, Bend, Scissoring (CH ₂) (CH ₃) (-CH ₂ CH ₃)	(1)
1446	0.26	-	-	Deformation, Bend (CH ₂)	(1)
1447	0.2	-	-	Deformation, Bend (CH ₂)	(1)
1448	0.13	0.00	-	Deformation (CH ₂) (-CH ₂ CH ₃)	(1)
1449	0.05	0.09	-	Lipids, Vibration (CH)	(1)
1450	-	0.18	-	Deformation, Bend (CH ₂) (CH)	(1)
1451	-	0.26	-	Deformation (-CH ₂ CH ₃)	(1)
1454	-	0.27	-	Overlapping Asymmetric CH ₃ Bending and CH ₂ Scissoring	(1)
1460	-	0.21	-	Deformation (CH ₂) (CH ₃)	(1)
1465	-	0.40	-	Lipids	(1)
1525	-	0.27	-	Vibration (C=C)	(1)
1554	0.38	-	0.79	Discovered in this research	
1580	-	-	0.54	Stretching (C-C)	(1)
1582	0.06	-	0.60	Discovered in this research	
1585	0.16	-	0.65	Olefinic Stretch (C=C)	(1)
1607	0.60	-	0.82	Discovered in this research	
1616	0.68	-	0.79	Discovered in this research	
1628	-	0.13	0.30	Stretch (C=C)	(1)
1652	-	0.38	-	Lipids, Stretch (C=C)	(1)
1655	-	0.40	-	Lipids, Stretch (C=C) (C=O)	(1)
1656	-	0.37	-	Lipids, Stretch, <i>cis</i> Vibration (C=C)	(1)
1657	-	0.32	-	Fatty Acids	(1)

1660	-	0.13	-	Lipids, Fatty Acids, <i>cis</i> Vibration (C=C)	(1)
1667	-	0.11	-	Stretch (C=C) (C=O)	(1)
1674	-	0.54	-	Stretch (C=C)	(1)
1734	-	0.21	-	Lipids, Stretch (C=O)	(1)
1736	-	0.18	-	Lipids, Esters (C=O)	(1)
1738	-	0.27	-	Lipids	(1)
1744	-	-	-	Lipids (Carbonyl Group)	(1)
1745	0.1	-	0.11	Lipid Assignment, Phospholipids, Vibration (C=O)	(1)
1746	0.21	-	0.31	Lipids, Stretch (C=O)	(1)
1747	0.22	-	0.42	Lipids (C=O)	(1)
1750	-	-	0.10	Lipids, Fatty Acids (C=C) (C=O)	(1)
1754	-	0.31	-	Lipids (C=O)	(1)
2817	-	0.37	-	Symmetric Stretch (-CH ₂)	(1)
2840	-	0.49	-	Symmetric Stretch (-CH ₃)	(1)
2850	-	0.19	0.10	Lipids, Fatty Acids, Symmetric Stretch (-CH ₂) (-CH ₃)	(1)
2855	0.52	-	0.53	Symmetric Stretch (-CH ₂) (-CH ₃), Asymmetric Stretch (-CH ₂)	(1, 2)
2856	0.59	-	0.58	Symmetric Stretch (-CH ₂) (-CH ₃), Asymmetric Stretch (-CH ₂)	(1, 2)
2858	0.60	-	0.60	Symmetric Stretch (-CH ₂) (-CH ₃), Asymmetric Stretch (-CH ₂)	(1, 2)
2859	0.55	-	0.56	Symmetric Stretch (-CH ₂) (-CH ₃), Asymmetric Stretch (-CH ₂)	(1, 2)
2868	0.76	-	0.63	Symmetric Stretch (-CH ₂) (-CH ₃), Asymmetric Stretch	(1)

				(-CH ₂)	
2870	0.78	-	0.64	Symmetric Stretch (-CH ₂) (-CH ₃), Asymmetric Stretch (-CH ₂)	(1, 6)
2876	0.67	-	0.47	Stretch (-CH), Asymmetric Stretch (-CH ₂)	(1)
2883	-	0.27	-	Stretch (-CH), Asymmetric Stretch (-CH ₂)	(1)
2889	-	0.17	0.22	Asymmetric Stretch (-CH ₂)	(1)
2893	-	0.20	0.21	Symmetric Stretch (-CH ₃)	(1)
2900	-	0.51	-	Stretch (-CH)	(1)
2910	0.67	-	0.52	Stretch (-CH ₃)	(1)
2915	0.74	-	0.47	Lipids (-CH)	(1)
2924	0.76	-	0.60	Asymmetric Stretch (-CH ₂)	(6, 7)
2930	0.79	-	0.50	Asymmetric Stretch (-CH)	(2)
2933	0.81	-	0.47	Asymmetric Stretch (-CH)	(1)
2940	0.62	-	0.38	Vibration (-CH) (-CH ₂)	(1)
2954	-	0.23	-	Asymmetric Stretch (-CH ₃)	(6)
2956	-	0.51	-	Asymmetric Stretch (-CH ₃)	(1)
2960	-	0.65	-	Asymmetric Stretch (-CH ₃)	(1)
2970	-	0.55	-	Asymmetric Stretch (-CH ₃)	(1, 5)
3008	-	0.52	-	Lipids, Fatty Acids, Asymmetric Stretch (=CH)	(1)
3010	-	0.36	-	Unsaturated Fatty Acids, Stretch (=CH)	(1)
3015	0.04	0.08	-	Lipids, Vibration (=CH)	(1)

* Negative correlation coefficients are represented by a dash.

Supplementary Table 2. All literature cited Raman bands considered for each amino acid (8, 9) for compiling the optimized results shown in Table 1.

Amino Acids	Associated Raman Bands (cm⁻¹)
Ala	399, 533, 771, 852, 922, 1021, 1115, 1149, 1308, 1361, 1378, 1411, 1464, 1485, 1599
Arg	857, 873, 930, 985, 1036, 1086, 1176, 1199, 1264, 1310, 1317, 1408, 1423, 1443, 1477
Asp/Asn	749, 779, 877, 939, 1084, 1125, 1338, 1408, 1426, 1695
Cys	455, 499, 542, 613, 678, 785, 873, 967, 1341, 1385, 1410
Glu/Gln	623, 669, 873, 917, 988, 1080, 1182, 1319, 1379, 1422, 1462, 1637, 1682
Gly	359, 496, 603, 898, 1036, 1327, 1332, 1413, 1442, 1458, 1515
His	539, 657, 731, 784, 806, 854, 919, 929, 964, 977, 1062, 1088, 1113, 1176, 1225, 1252, 1272, 1319, 1336, 1349, 1355, 1409, 1431, 1443, 1500, 1578
Ile	536, 557, 675, 765, 820, 825, 852, 873, 918, 964, 993, 1017, 1033, 1134, 1168, 1191, 1257, 1309, 1329, 1338, 1355, 1398, 1413, 1421, 1448, 1465, 1514, 1583, 1619
Leu	771, 836, 848, 1135, 1178, 1187, 1243, 1342, 1410, 1457, 1583, 1623
Lys	625, 785, 849, 877, 912, 946, 975, 988, 1036, 1055, 1064, 1072, 1097, 1141, 1168, 1305, 1321, 1341, 1360, 1399, 1414, 1433, 1447, 1456, 1464, 1485, 1609
Met	645, 682, 700, 721, 765, 805, 877, 1068, 1175, 1245, 1265, 1321, 1343, 1355, 1415, 1428, 1447
Phe	469, 525, 606, 622, 748, 821, 835, 915, 954, 1005, 1036, 1158, 1188, 1214, 1310, 1413, 1608
Pro	642, 843, 899, 921, 986, 1035, 1084, 1239, 1453
Ser	610, 805, 814, 854, 857, 969, 1010, 1127, 1220, 1301, 1327, 1417, 1468
Thr	447, 564, 872, 932, 1045, 1116, 1251, 1341, 1410, 1419, 1458
Trp	756, 759, 849, 875, 961, 1010, 1013, 1078, 1120, 1233, 1253, 1340, 1360, 1426, 1460, 1489, 1559
Tyr	641, 798, 831, 847, 986, 1044, 1180, 1201, 1267, 1328, 1615
Val	542, 665, 753, 776, 825, 850, 902, 948, 965, 1000, 1066, 1100, 1126, 1200, 1273, 1300, 1331, 1354, 1396, 1400, 1427, 1454, 1467, 1509

Supplementary Table 3. Alternative Raman band assignment Set 1 for amino acids. Correlation coefficient (R) between Raman and UPLC data = 0.86.

Amino Acids	Optimum Raman Band (cm⁻¹)	Amino Acid Composition Determined by Raman	Amino Acid Composition Determined by UPLC	Published Amino Acid Composition
Ala	1308	11.4	11.1	9.60
Arg	1423	3.53	3.39	5.53
Asp/Asn	1338	11.9	8.98	9.01
Cys	785	4.47	1.64*	1.64
Glu/Gln	1637	6.75	9.16	9.84
Gly	1327	10.9	12.0	11.5
His	854	2.01	0.890	1.77
Ile	1329	11.2	8.76	5.43
Leu	1457	11.7	11.4	8.42
Lys	1168	2.19	5.24	6.41
Met	877	1.63	0.467	2.87
Phe	1413	3.05	4.59	3.46
Pro	843	0.749	1.02	4.13
Ser	1010	4.50	4.42	4.03
Thr	1116	3.48	6.26	4.74
Trp	1426	3.68	1.06*	1.06
Tyr	641	1.69	0.390	2.58
Val	1126	5.20	9.22	7.91
Sum	-	100	100	100

* Values for Cys and Trp could not be resolved by the UPLC method. Cys is converted to several oxidation products, while Trp is destroyed during acidic protein hydrolysis used in this research. The published literature values were used for Cys and Trp.

** Amino acid composition is defined as the fraction of each amino acid in *E. coli* total protein.

*** Possible overlapping bands: Gly (1327 cm⁻¹) / Ile (1329 cm⁻¹); Arg (1423 cm⁻¹) / Trp (1426 cm⁻¹)

Supplementary Table 4. Alternative Raman band assignment Set 2 for amino acids. Correlation coefficient (R) between Raman and UPLC data = 0.89.

Amino Acids	Optimum Raman Band (cm⁻¹)	Amino Acid Composition Determined by Raman	Amino Acid Composition Determined by UPLC	Published Amino Acid Composition
Ala	1308	12.0	11.1	9.60
Arg	1199	2.67	3.39	5.53
Asp/Asn	1338	12.5	8.98	9.01
Cys	499	1.57	1.64*	1.64
Glu/Gln	1637	7.10	9.16	9.84
Gly	1442	10.7	12.0	11.5
His	964	1.89	0.890	1.77
Ile	1355	7.03	8.76	5.43
Leu	1457	12.3	11.4	8.42
Lys	1064	2.55	5.24	6.41
Met	1068	3.13	0.467	2.87
Phe	1413	3.21	4.59	3.46
Pro	843	0.787	1.02	4.13
Ser	1417	3.46	4.42	4.03
Thr	1116	3.66	6.26	4.74
Trp	849	1.57	1.06*	1.06
Tyr	641	1.77	0.390	2.58
Val	1331	12.1	9.22	7.91
Sum	-	100	100	100

* Values for Cys and Trp could not be resolved by the UPLC method. Cys is converted to several oxidation products, while Trp is destroyed during acidic protein hydrolysis used in this research. The published literature values were used for Cys and Trp.

** Amino acid composition is defined as the fraction of each amino acid in *E. coli* total protein.

*** Possible overlapping bands: Pro (843 cm⁻¹) / Trp (849 cm⁻¹); Lys (1064 cm⁻¹) / Met (1068 cm⁻¹); Val (1331 cm⁻¹) / Asp/Asn (1338 cm⁻¹)

Supplementary Table 5. Alternative Raman band assignment Set 3 for amino acids. Correlation coefficient (R) between Raman and UPLC data = 0.85.

Amino Acids	Optimum Raman Band (cm⁻¹)	Amino Acid Composition Determined by Raman	Amino Acid Composition Determined by UPLC	Published Amino Acid Composition
Ala	1464	10.5	11.1	9.60
Arg	1036	1.97	3.39	5.53
Asp/Asn	1426	3.83	8.98	9.01
Cys	1385	4.08	1.64*	1.64
Glu/Gln	1319	11.6	9.16	9.84
Gly	1332	12.2	12.0	11.5
His	964	1.87	0.890	1.77
Ile	1257	8.66	8.76	5.43
Leu	1457	12.2	11.4	8.42
Lys	785	4.65	5.24	6.41
Met	765	1.22	0.467	2.87
Phe	1214	6.20	4.59	3.46
Pro	986	2.38	1.02	4.13
Ser	805	1.73	4.42	4.03
Thr	1116	3.63	6.26	4.74
Trp	1078	4.42	1.06*	1.06
Tyr	798	1.82	0.390	2.58
Val	1354	7.10	9.22	7.91
Sum	-	100	100	100

* Values for Cys and Trp could not be resolved by the UPLC method. Cys is converted to several oxidation products, while Trp is destroyed during acidic protein hydrolysis used in this research. The published literature values were used for Cys and Trp.

** Amino acid composition is defined as the fraction of each amino acid in *E. coli* total protein.

*** Possible overlapping bands: Tyr (798 cm⁻¹) / Ser (805 cm⁻¹)

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