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Supplementary Information

Illuminating the Biochemical Interaction of Antimicrobial Few-Layer Black Phosphorus with Microbial Cells Using Synchrotron macro-ATR-FTIR

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Hierarchical Cluster Analysis of the ATR-FTIR Spectra

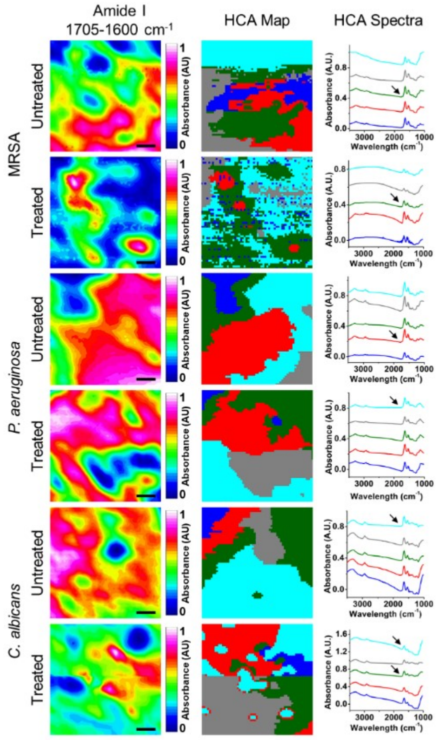

Figure S1 – HCA. High-resolution ATR-FTIR spectral maps integrated for the Amide I region (left). Resulting HCA cluster map (middle) and corresponding spectra (right). The black arrow indicates the cluster used.

For the MRSA samples, there are noticeable “dots” appearing in the spectral maps. This can be attributed to hydrogen interference indicating the samples had not fully dried before imaging. For the treated MRSA sample, the HCA analysis differentiated a majority of the spectra with the hydrogen interference into one analysis clusters. The cluster selected for the untreated MRSA sample also had the least visible interference from the dotting. The number of spectra associated with the selected *C. albicans* spectra was relatively small when compared to the control spectra, so two averages were combined for the PCA analysis.

Principle Component Analysis of the ATR-FTIR Spectra

For MRSA, *P. aeruginosa* and *C. albicans* the PC-3 score plot and loading spectra (**Figure S2**) showed patterns to PC-2. For the scores plots, most of the variation in PC-3 was 5% for MRSA and 1% for *P. aeruginosa* and *C. albicans,* which indicate the changes identified are minor. The loading spectra peaks show similar changes as highlighted by the PC-2 spectra. A majority of the bands are within the amide I and II bands,[1-4] further highlighting how the cellular proteins were the most impacted by the presence of BP nanoflakes. A summary of the key PC-3 peaks for all cells are shown in **Table S1**, and the corresponding biochemical compound were assigned in **Table S2-S4**.

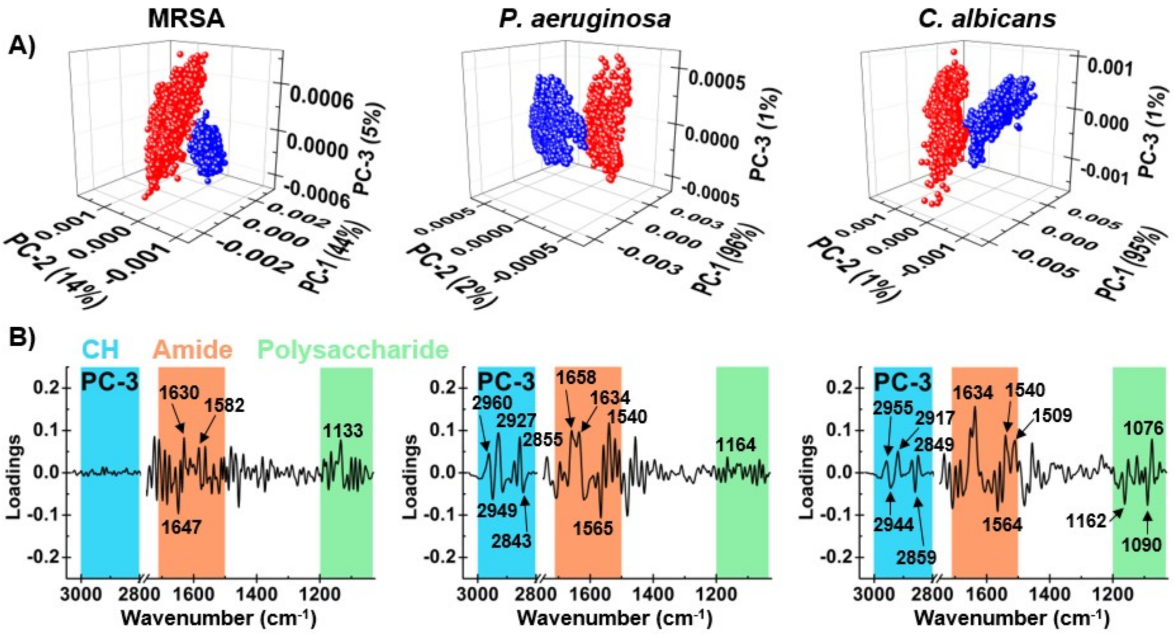

Figure S2. PCA score plots of the untreated and treated groups for each microbial cell. Comparison between PC-1 and PC-2 scores for MRSA, *P. aeruginosa* and *C. albicans* cells. The blue dots are the untreated sample, and the red dots are the BP treated samples.

Table S1. Summary of PC3 loading peaks

Cell	*Wavelength (cm^{-1})*	*Assignment*	*Ref.*
MRSA	1647-1630	Amide I protein structures (unordered, α-helix or β-sheet	3, 5, 6
	1582	Amide II protein structures (base rings or α-helix)	1, 4, 5
	1133	Polysaccharide (C-OH or O-P-O stretching)	5, 6
P. aeruginosa	2960-2843	C-H stretching of CH_2 and CH_3 groups in cellular proteins, nucleic acids and lipids	2, 4, 5
	1658-1634	Amide I protein structures (loop, α-helix or β-sheet)	1, 2, 5
	1565-1540	Amide II protein structures (base rings or β-sheet)	5, 6
	1164	Polysaccharides (C-C, C-OH or CO stretching)	
C. albicans	2955-2849	C-H stretching of CH_2 and CH_3 groups in cellular proteins, nucleic acids and lipids	2, 4, 5
	1634	Amide I protein structures (β-sheet)	1, 2, 5
	1564-1509	Amide II protein structures (base rings or β-sheet)	5, 6
	1162-1076	Oxidative damage to polysaccharide or nucleic acid	2, 5, 6

Table S2. Peak assignment from the PCA loading for MRSA.

PC	*Region*	*Wavenumber (cm^{-1})*	*Proposed band assignment*	*reference*
1+	Lipid (C-H, N-H)	2945	v_s(C-H) of the CH_2 groups in the lipids	5, 7
		2864	C-H stretching in the lipids	5
	Amide I (C=O, C=C, C-N, N-H)	1616	Protein base ring stretching vibrations of the phenyl group	5
	Amide II (C-C, C=C, C-H, C-N, N-H)	1577	Protein base ring stretching vibrations of the phenyl group	5
		1560	Protein base ring stretching vibrations with little interaction with CH in-plane bending	5
		1540	Protein β-sheet structure	5, 6
		1520	Protein structure (N-H)	1, 5
		1507	Protein in-plane CH bending vibration from the phenyl rings	5
1-	Lipid (C-H, N-H)	2958	v_{as}(C-H) of the CH_3 groups of the from cellular proteins, nucleic acids and lipids	2, 5
		2925	v_{as} (C-H) of the CH_2 groups in the lipids	1, 2, 4, 5
		2873	v_s(C-H) of the CH_3 group of the acyl chains in the lipids	2, 4, 5
		2845	v_s (C-H) of methoxy group	5, 7
	Amide I (C=O, C=C, C-N, N-H)	1660	Protein C=C *cis* stretching in turn and coil structures	3, 5

		1642	Protein β-sheet structure C_5 methylated cytosine	3, 5, 6
	Amide II (C-C, C=C, C-H, C-N, N-H)	1549	Protein α-helix and β-sheet structures (perpendicular modes)	2, 5, 6
		1531	Protein aggregated β-sheet structures	1, 4, 5
		1513	Protein α-helix structure (parallel) Possible presence of tyrosine nucleic acid	2, 4, 5
	Polysaccharide (C-OH O-P-O)	1135	C-OH stretching band	5
2+	Lipid (C-H, N-H)	2960	v_{as}(C-H) of the CH_3 groups in the lipids	2, 4, 5
		2924	v_{as} (C-H) of the CH_2 groups in the lipids	1, 2, 4, 5
		2851	v_s(C–H) from acyl chains and aliphatic alkanes of the lipids (cholesterol, phospholipid and creatine)	1, 2, 4, 5, 7
	Amide I (C=O, C=C, C-N, N-H)	1637	Protein β-sheet structure and 3_{10} helices O-Hydroxy and O-amino-aryl aldehydes	1, 3, 5, 6
	Amide II (C-C, C=C, C-H, C-N, N-H)	1558	Protein α-helix and β-sheet (antiparallel) structures Ring stretching with little interaction with CH in-plane bending (base vibrations)	5, 7
		1541	Protein structure	5, 6
		1508	Protein phenyl rings in-plane CH bending vibration	5
	Polysaccharide (C-OH O-P-O)	1134	Oligosaccharide C-OH stretching band Symmetric stretching PO_2^-	5, 6
3+	Amide I (C=O, C=C, C-N, N-H)	1647	Protein structures (unordered, α-helix or β-sheet) C_5 methylated cytosine	3, 5, 6
	Amide II (C-C, C=C, C-H, C-N, N-H)	1582	Protein α-helix structure Protein base ring stretching vibrations of the phenyl group NH_2^+ and CO_2 (carboxylic acid)	1, 4, 5
	Polysaccharide (C-OH O-P-O)	1133	C-OH stretching band v_s (PO_2^-)	5, 6
3-	Amide I (C=O, C=C, C-N, N-H)	1630	Protein aggregated β-sheet structure	1, 3-5

Table S3. Peak assignment from the PCA loading for *P. aeruginosa*.

PC	*Region*	*Wave Number (cm^{-1})*	*Proposed band assignment*	*reference*
1+	Lipid (C-H, N-H)	2945	v_s(C-H) of the CH$_2$ groups in the lipids	[5, 7]
		2864	C-H stretching in the lipids	[5]
	Amide I (C=O, C=C, C-N, N-H)	1616	Protein base ring stretching vibrations of the phenyl group	[5]
	Amide II (C-C, C=C, C-H, C-N, N-H)	1577	Protein base ring stretching vibrations of the phenyl group	[5]
		1560	Protein base ring stretching vibrations with little interaction with CH in-plane bending	[5]
		1540	Protein β-sheet structure	[5, 6]
		1520	Protein structure (N-H)	[5]
		1507	Protein in-plane CH bending vibration from the phenyl rings	[5]
	Polysaccharide (C-H)	1181	C-H stretching in CH$_2$ group	[5]
1-	Lipid (C-H, N-H)	2958	v_{as}(C-H) of the CH$_3$ groups of the from cellular proteins, nucleic acids and lipids	[2, 5]
		2925	v_{as} (C-H) of the CH$_2$ groups in the lipids	[1, 2, 4, 5]
		2873	v_s(C-H) of the CH$_3$ group of the acyl chains in the lipids	[2, 4, 5]
		2845	v_s (C-H) of methoxy group	[5, 7]
	Amide I (C=O, C=C, C-N, N-H)	1660	Protein C=C *cis* stretching in turn and coil structures	[3, 5]
		1642	Protein β-sheet structure C$_5$ methylated cytosine	[3, 5, 6]
	Amide II (C-C, C=C, C-H, C-N, N-H)	1549	Protein α-helix and β-sheet structures (perpendicular modes)	[2, 5, 6]
		1531	Protein aggregated β-sheet structures	[1, 4, 5]
		1513	Protein α-helix structure (parallel) Possible presence of tyrosine nucleic acid	[2, 4, 5]
2+	Lipid (C-H, N-H)	2955	C-H stretching in the lipids	[5]
		2808	C-H stretching of the CH$_2$ and CH$_3$ groups in lipids (cholesterol, phospholipid or creatine) Possible N-H stretch	[5]
	Amide I	1654	Protein α-helix structure	[1, 2, 4, 5]

	(C=O, C=C, C-N, N-H)		C=O, C=N, N-H of adenine, thymine, guanine, cytosine	
	Amide II (N-H, C-N, N-C-H)	1544	Protein α-helix structure	4-6
	Polysaccharide (C-OH O-P-O)	1132	C-OH stretching band v_s (PO_2^-)	5, 6
2-	Lipid (C-H, N-H)	2930	v_{as}(C-H) of the CH_2 groups in the lipids	1, 5
		2854	v_s(C-H) of the CH_2 group in the lipids	2, 4, 5
	Amide I (C=O, C=C, C-N, N-H)	1642	Protein β-sheet structure C_5 methylated cytosine	3, 5, 6
3+	Lipid (C-H, N-H)	2960	v_{as}(C-H) of the CH_3 groups in the lipids	2, 4, 5
		2927	v_{as}(C-H) of the CH_2 groups in the lipids	1, 2, 5, 7
		2855	v_s(C-H) or v_{as}(C-H) of the CH_2 group in the lipids	4, 5
	Amide I (C=O, C=C, C-N, N-H)	1658	Protein structures (loop or α-helix) C=O, C=C, NH2 in uracyl and guanine	1, 2, 4, 5
		1634	Protein β-sheet structure O-Hydroxy and O-amino-aryl aldehydes	1, 2, 5
	Amide II N-H, C-N, N-C-H)	1540	Protein β-sheet structure	5, 6
	Polysaccharide (C-O, C-OH, C-C)	1164	C-O groups in proteins v(C-C), d(C-OH), v(CO) stretching	5
3-	Lipid (C-H, N-H)	2949	v_s(C-H) of the CH_2 groups in the lipids	5, 7
		2843	v_s (C-H) of methoxy group	5
	Amide II (C-C, C-H, C-N)	1565	Protein base ring stretching vibrations with little interaction with CH in-plane bending	5

Table S4. Peak assignment from the PCA loading for C. *albicans.*

PC	*Region*	*Wave Number (cm^{-1})*	*Proposed band assignment*	*reference*
1+	Lipid (C-H, N-H)	2945	v_s(C-H) of the CH_2 groups in the lipids	5, 7
		2864	C-H stretching in the lipids	5

1-	Amide I (C=O, C=C, C-N, N-H)	1616	Protein base ring stretching vibrations of the phenyl group	5
	Amide II (C-C, C=C, C-H, C-N, N-H)	1577	Protein base ring stretching vibrations of the phenyl group	5
		1560	Protein base ring stretching vibrations with little interaction with CH in-plane bending	5
		1540	Protein β-sheet structure	5, 6
		1520	Protein structure (N-H)	5
		1507	Protein in-plane CH bending vibration from the phenyl rings	5
	Polysaccharide (C-H, C-O)	1183	C-H stretching in CH_2 group C-O (P-sub benzene and propionates and higher esters)	1, 5
	Lipid (C-H, N-H)	2958	v_{as}(C-H) of the CH_3 groups of the from cellular proteins, nucleic acids and lipids	2, 5
		2925	v_{as} (C-H) of the CH_2 groups in the lipids	1, 2, 4, 5
		2873	v_s(C-H) of the CH_3 group of the acyl chains in the lipids	2, 4, 5
		2845	v_s (C-H) of methoxy group	5, 7
	Amide I (C=O, C=C, C-N, N-H)	1660	Protein C=C *cis* stretching in turn and coil structures	3, 5
		1642	Protein β-sheet structure C_5 methylated cytosine	3, 5, 6
	Amide II (C-C, C=C, C-H, C-N, N-H)	1549	Protein α-helix and β-sheet structures (perpendicular modes)	2, 5, 6
		1531	Protein aggregated β-sheet structures	1, 4, 5
		1513	Protein α-helix structure (parallel) Possible presence of tyrosine nucleic acid	2, 4, 5
	Polysaccharide (C-O, C-C, C-O-H)	1151	v_{as} (CO-O-C) of glycogen and nucleic acid (DNA)	2, 5, 7
2+	Amide I (C=O, C=C, C-N, N-H)	1621	Protein antiparallel β-sheet structure Nucleic acids (base carbonyl stretching and ring breathing mode)	5, 7
	Polysaccharide (C-O, C-C, C-O-H)	1151	v_{as} (CO-O-C) of glycogen and nucleic acid (DNA)	2, 5, 7
		1076	Skeletal *cis*(C-C) conformation of DNA v_s (C-O-C) and v_s (C-C),	5, 6

			v_s (PO2-) and v_s (PO3^{2-}) (oxidative damage to DNA)	
2-	Lipid (C-H, N-H)	2932	v_{as}(C-H) of the CH_2 groups in the lipids	4, 5
		2958	C-H stretching in the lipids	5
	Amide I (C=O, C=C, C-N, N-H)	1641	Protein structure Deformation of water (H-O-H) C_5 methylated cytosine	5, 6
	Amide II (N-H, C-N)	1522	Protein structure (N-H)	1, 5
	Polysaccharide (C-O, C-OH, C-C)	1164	C-O groups in proteins v(C-C), d(C-OH), v(CO) stretching	5
	Polysaccharide (C-O-P, O-P-O)	1091	v_s (PO_2^-)	2, 5, 6
	Nucleic acid C-O, C-O-C, O-P-O	1068	C-O stretching in nucleic acids (DNA) C-O-C stretching in phospholipids v_s (R-O-P-O-R') from ring vibrations in carbohydrates	2, 5
3+	Lipid (C-H, N-H)	2955	C-H stretching in the lipids	5
		2917	C-H stretching of the CH_2 and CH_3 groups in the lipids (cholesterol, phospholipid and creatine)	5
		2849	V_s (C-H) of CH_2 of the lipids (Cholesterol, phospholipid and creatine)	1, 5, 7
	Amide I (C=O, C=C, C-N, N-H)	1634	Protein β-sheet structure O-Hydroxy and O-amino-aryl aldehydes	1, 2, 5
	Amide II (C-C, C-H, C-N)	1540	Protein β-sheet structure	5, 6
		1509	Protein in-plane CH bending vibration from the phenyl rings	5
	Nucleic acid (C-C, O-P-O)	1076	Skeletal *cis*(C-C) conformation of DNA v_s (C-O-C) and v_s(C-C), v_s (PO2-) and v_s (PO3^{2-}) (oxidative damage to DNA)	5, 6
3-	Lipid (C-H, N-H)	2944	C-H stretching in the lipids	5
		2859	C-H stretching in the lipids	5
	Amide II (C-C, C-H, C-N)	1564	Protein base ring stretching vibrations with little interaction with CH in-plane bending	5
	Polysaccharide (C-O, C-OH, C-C, C-O-C)	1162	C-O groups in proteins v(C-C), d(C-OH), v(CO) stretching d(C-O-C) cellulose/polysaccharide ring	5, 7

	Polysaccharide (O-P-O)	1090	$v_s\,(PO_2^-)$	2, 5, 6

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