

Table S1 SERS spectral peak assignments of *Bacillus subtilis* biofilm.

SERS peak position	SERS peak assignments	References
568 cm ⁻¹	Hypoxanthine (DNA)	1
624 cm ⁻¹	Aromatic ring skeleton (carbohydrates)	2
653 cm ⁻¹	Ring vibration in polydeoxyadenosine (DNA)	3
675 cm ⁻¹	Guanosine (DNA)	1
726 cm ⁻¹	Glycosidic ring mode of adenine (DNA)	2
798 cm ⁻¹	Ring stretching and breathing mode of Uracil (nucleic acid)	4
908 cm ⁻¹	C–O–C stretching of glycosidic linkage in saccharides	5
954 cm ⁻¹	C-N stretching of polyene chain in carbohydrate	5
1001 cm ⁻¹	breathing mode of symmetric ring (protein)	6
1093 cm ⁻¹	C-C gauche stretching in membrane lipid	7
1130 cm ⁻¹	C-N stretching in proteins	6 5
1243 cm ⁻¹	C=O stretching (amide III)	6
1329 cm ⁻¹	CH vibration (protein)	8
1450 cm ⁻¹	CH ₂ vibration (lipids)	9

Table S2 Some message about Raman peaks

Raman peaks	Message about Raman peaks
1329 cm ⁻¹ (C-H vibration) ⁸ , 1001 cm ⁻¹ (ring breathing mode) ⁶ , 1130 cm ⁻¹ (C-N stretching) ⁶ ⁵ and 1243 cm ⁻¹ (Amide III) ⁶	These Raman peaks are associated with proteins. After the exposure of antibiotic, peak at 1329 cm ⁻¹ shows a continuous decrease in intensity due to conformational changes occurring in protein molecules. Peaks at 1001 cm ⁻¹ , 1143 cm ⁻¹ and 1201 cm ⁻¹ disappear after the exposure of higher concentration of antibacterial agent, indicating the denaturation of proteins and cell death ⁸ .
1093 cm ⁻¹ (C-C stretching) ⁷	This peak is attributed to lipids. Raman peak at 1093 cm ⁻¹ position shows a decrease in intensity after the exposure of antibiotic due to high level of environmental stress ⁷ . Biofilm responds to stress by increasing the synthesis of lipids ¹⁰ .
653 cm ⁻¹ (polydeoxyadenosine ring's vibration) ³ , 568 cm ⁻¹ (hypoxanthine) ¹ , and 798 cm ⁻¹ (ring stretching and breathing mode of Uracil (nucleic acid) ⁴	These peaks are attributed to genetic material (DNA). After the exposure of antibiotic, Raman peak at 653 cm ⁻¹ shows a decrease in intensity as concentration of lab synthesized antibacterial agent is increased. This decrease indicates the destruction of nucleic acid in <i>Bacillus subtilis</i> biofilm ³ .
908 cm ⁻¹ (C–O–C stretching of glycosidic linkage in saccharides) ⁵ , 954 cm ⁻¹ (C-N stretching of polyene chain in carbohydrate) ⁵ and 624 cm ⁻¹ (Aromatic ring skeleton) ²	These peaks are associated to carbohydrates and shows the decrease in intensity after the exposure of antibacterial agent due to the change in carbohydrate's content in <i>Bacillus subtilis</i> biofilm ⁵ .