FT-IR analysis of pathogen inactivation by middle IR femtosecond laser pulses

Irina N. Saraeva[1], Svetlana N. Shelygina[1], Alena A. Nastulyavichus[1], Eteri R. Tolordava[1,2], Roman A. Khmelnitskii[1], S. I. Kudryashov[1]

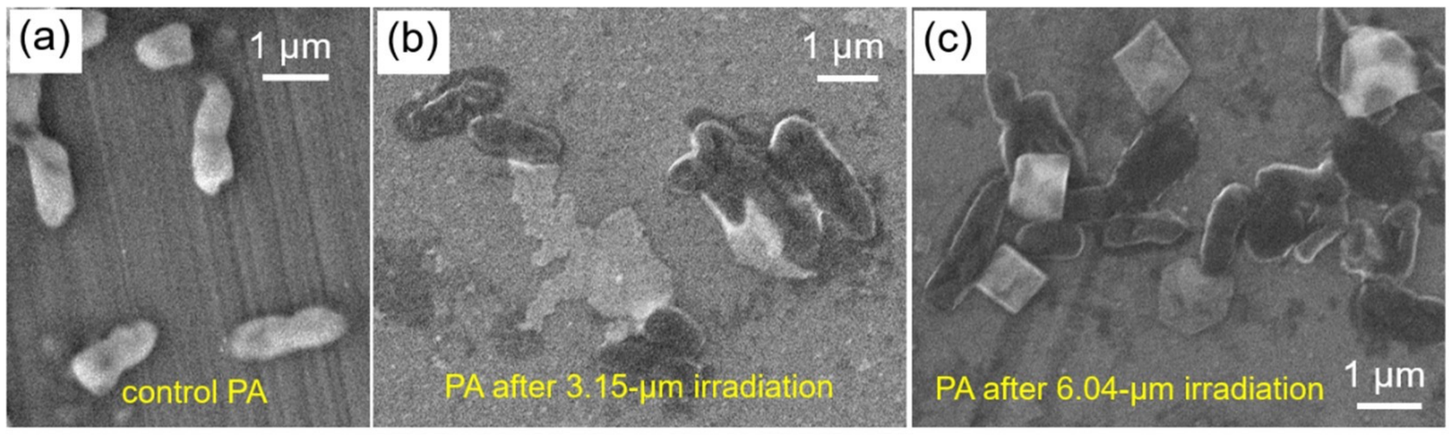
[1]Lebedev Physical Institute RAS, Moscow, Russia; [2]Gamaleya National Research Center for Epidemiology and Microbiology, Moscow, Russia

e-mail: shelyginasn@lebedev.ru

ABSTRACT

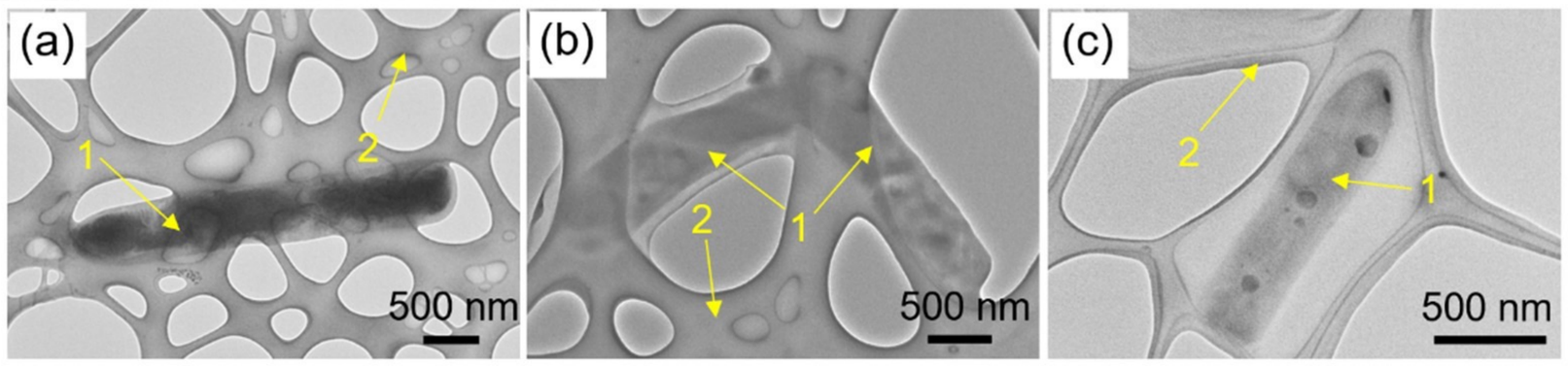
We report the successful inactivation of *P. aeruginosa* bacteria by femtosecond infrared laser radiation at the resonant wavelengths of 3.15 μm and 6.04 μm, chosen due to the presence of characteristic molecular vibrations in the main structural elements of the bacterial cells in these spectral ranges: vibrations of amide groups in proteins (1500–1700 cm^{-1}), and C-H vibrations in membrane proteins and lipids (2800–3000 cm^{-1}). The underlying bactericidal structural molecular changes were revealed by the FT-IR spectroscopy, with the spectral peaks parameters being obtained by Lorentzian fitting with the hidden peaks revealed by the second derivative calculations, while no visible damage to the cell membranes was identified by scanning and transmission electron microscopy.

SEM AND TEM CHARACTERIZATION



SEM images of *P. aeruginosa* dried on a Si substrate.

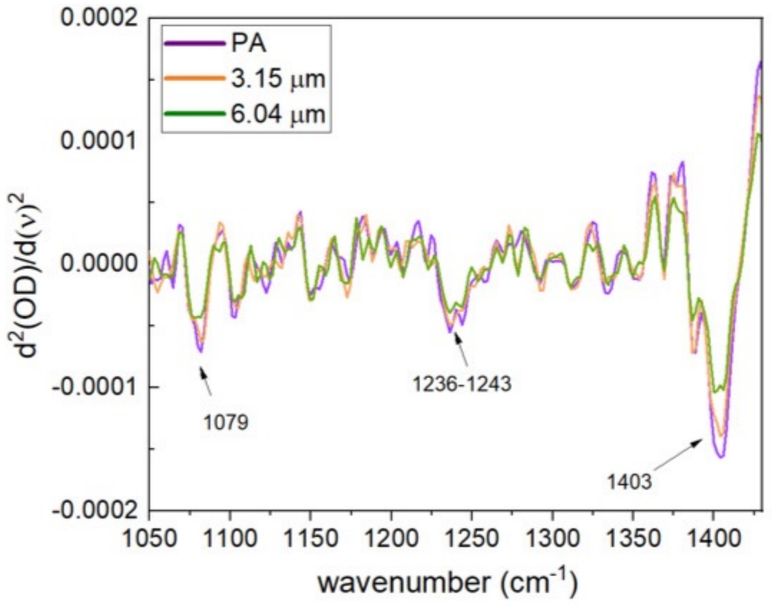
SEM and TEM analysis illustrates the due to the coincidence with the characteristic vibrations of the main structural elements unchanged integrity of the cell membrane, therefore, suggesting the intermolecular change in the bacterial cells: vibrations of amide groups of proteins (1500–1700 cm^{-1}) and in bacterial cells.

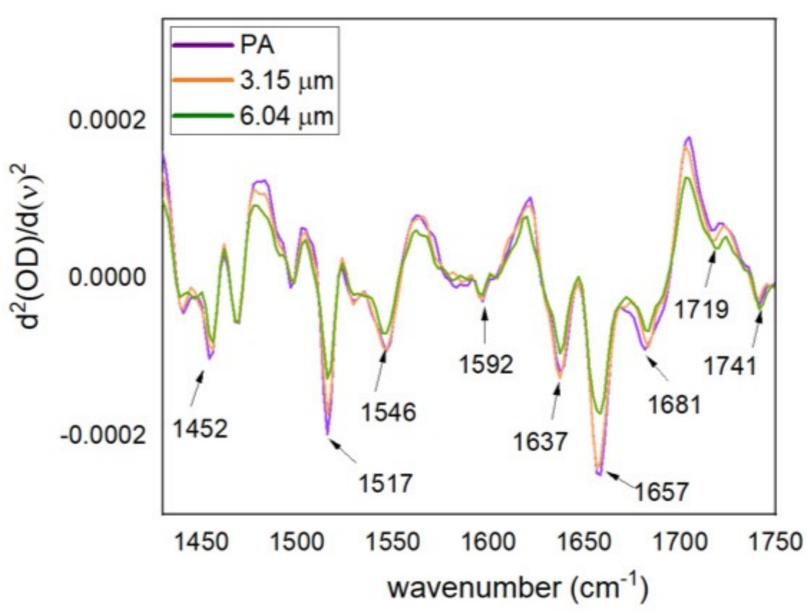


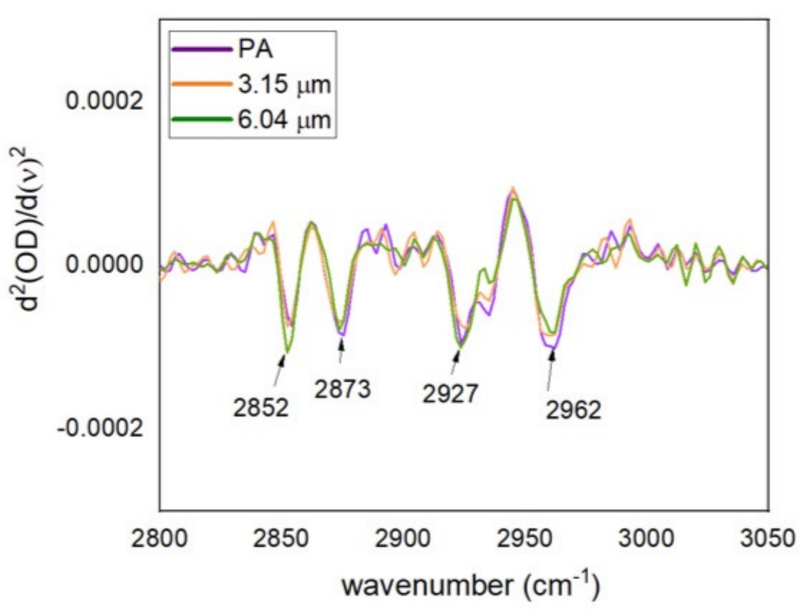
TEM images of *P. aeruginosa* cells, washed out from the CaF_2 substrate. 1-bacterial cells; 2-the carbon coating on the Au grid used in the TEM analysis.

FT-IR SPECTRAL ANALYSIS

Selected regions of second-derivative FT−IR spectra of *P. aeruginosa* on the CaF_2 before and after 3.15-μm and 6.04-μm laser irradiation







Functional Groups	Frequency (cm^{-1}) Bandwidth (a.u.) Area (a.u.)		
	Control	3.15 μm exposure	6.04 μm exposure
PO_2 str (sym) of nucleic acids and PO_2	1079.97 163.82 11.01	1079.97 123.37 6.76	1079.97 177.61 11.04
P=O str (asym) of phosphodiesters	1240.04 52.95 1.88	1240.04 53.28 1.67	1240.04 53.35 1.34
COO-	1403.97 48.13 4.13	1402.04 45.25 3.29	1402.04 47.21 2.75
C-H def of CH2	1450.25 32.97 1.33	1450.25 40.19 1.61	1450.25 40.21 1.32
Amide II N-H bend, C-N str of proteins	1544.75 48.19 3.95	1544.75 52.94 3.50	1542.82 58.79 3.06
Amide I ß-pleated sheets	1637.32 51.26 7.21	1637.32 47.80 5.76	1637.32 48.61 4.59
Amide I a-helices	1658.53 34.06 4.43	1658.53 34.86 4.42	1658.53 34.45 3.33
Amide I ß-pleated sheets	1681.67 29.08 2.84	1681.67 28.60 2.41	1681.67 27.87 1.82
C-H str (sym) of CH_2 in fatty acids	2852.29 32.79 0.43	2852.29 22.25 0.18	2852.29 11.11 0.06
C-H str (sym) of CH CH_3 str (sym) of mainly proteins	2873.51 27.65 0.51	2873.51 30.36 0.53	2875.43 69.43 1.27
C-H str (asym) in CH_2 of mainly lipids	2927.50 34.59 1.19	2927.50 36.56 1.20	2925.58 42.66 1.43

RESULTS

We confirm the more pronounced effect of the 6.04 μm laser radiation on membrane proteins, lipids, and fatty acids in *P. aeruginosa* bacteria. The membrane lipopolysaccharides peak at ~1080 cm^{-1} in *P. aeruginosa* are also affected after the 6.04 μm laser treatment. The peak at 1236–1240 cm^{-1} in phosphodiester, phospholipids, lipopolysaccharide, nucleic acids, ribose in bacterial membrane, nucleoid, and ribosomes exhibit their broadening after the IR treatment at both these wavelengths. The peak at 1658 cm^{-1} broadens after the IR treatment, and its area decreases, which indicates the α-helices content decreasing after the IR irradiation.

This research was funded by the Ministry of Science and Higher Education of the Russian Federation (Ural Federal University Program of Development within the Priority-2030 Program).