Serbian Microscopy Society | Српско Друштво за Микроскопију

MCM2019

PROCEEDINGS

from the

14th MULTINATIONAL CONGRESS ON MICROSCOPY

September 15–20, 2019, Belgrade, Serbia

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SEPTEMBER 15–20, 2019, BELGRADE, SERBIA

TITLE:

Proceedings from the 14th Multinational Congress on Microscopy, September 15–20, 2019, Belgrade, Serbia

PUBLISHERS:

University of Belgrade, Institute for Biological
Research "Siniša Stanković", National Institute of Republic of Serbia

Serbian Society for Microscopy, Serbia

FOR PUBLISHERS:

Dr. Mirjana Mihailović
Dr. Jasmina Grbović Novaković

EDITORS:

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ISBN 978-86-80335-11-7

PRINT:

Knjigoveznica i kartonaža Grbović M. Milica, M. Gorkog 43, Beograd 11000, Serbia
30 e-copies

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The effects of ionizing radiation on the cell wall of microalgae *Chlorella sorokiniana* - TEM study

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1. Introduction

Microalgae are exposed to ionizing radiation from natural (*e.g.* radionuclides from soil and rocks) and anthropogenic sources (radioactive waste, nuclear power accidents, *etc*). However, there is a very limited amount of data on the mechanisms that microalgae employ in response/adaptation to radiation [1]. The aim of our study was to determine the effects of X-ray irradiation on the cell wall of *Chlorella sorokiniana*. Microalgal cell wall represents a dynamic multi-layer structure, which is both a barrier and the line of contact between unicellular algae and the surroundings [2].

2. Details of experiments

C. sorokiniana (CCAP 211/8K) culture was grown in 3N-BBM+V medium, at 22°C with a continuous photon flux of 120 µmol m^{-2} s^{-1} for 20 days, reaching stationary phase. Cultures were then exposed to X-rays at doses of 1, 2 and 5 Gy, left under the same conditions for additional 24 h, *C. sorokiniana* cells were collected by centrifugation (500 x g for 5 min) and fixed overnight at 4°C in 0.1M phosphate buffer (PB) (pH 7,2) containing 3% glutaraldehyde (SERVA, Germany) and 1% paraformalaldehyde (pH 6,9). Postfixation was performed with 1% osmium tetroxide (SERVA, Germany) in 0.1M PB (pH 7,2) for 2 h at room temperature. Samples were dehydrated in a graded acetone series and then embedded in resin for soft blocks (AGR1031, Agar Scientific, UK). Thin sections (0.07 mm), obtained with Leica UC7 ultramicrotome (Leica Microsystems, Germany), were stained with uranyl acetate and lead acetate and observed at 60 kV in a JEOL JEM-1010 TEM (JEOL, Japan). Characterisation of cell wall thickness was performed on TEM photographs using ImageJ (NIH). At least 25 cells with the nuclear mid-section for control and each examined dose were analysed on low (x7500) magnification. Thickness of the cell wall was measured at four points, (on x and y axis of the micrograph with 0 point at the cell's center), corresponding to the position of the small hand on the clock when it is set at 12h, 3h, 6h, 9h. In addition, gravimetry of isolated cell wall was performed. Isolation was conducted according to the previously described protocol that was slightly modified (starch was removed by amylase treatment of isolates) [3].

3. Results and discussion

TEM showed that cell wall of *C. sorokiniana* is composed of trilaminar sheath (TLS electron translucent line inserted between two electron dense lines; the outermost layer is a mature mother wall, while the thin inner layer is a daughter wall), and fibrilar cell wall. It is noteworthy that the obtained diameters for untreated microalgae were in accordance with available data [4]. The

analysis of TEM micrographs showed that there were no significant changes in the thickness of TLS for any of the used doses. However, the diameter of fibrillar wall was increased in response to irradiation for microalgae exposed to 1 Gy and 2 Gy. The thickness of cell wall in microalgae exposed to 5 Gy was not significantly different than in controls (Figure 1). A similar trend was observed by gravimetry of dry cell wall isolates normalized to biomass. It is important to note that no effects of radiation on biomass, at doses applied here, could be observed.

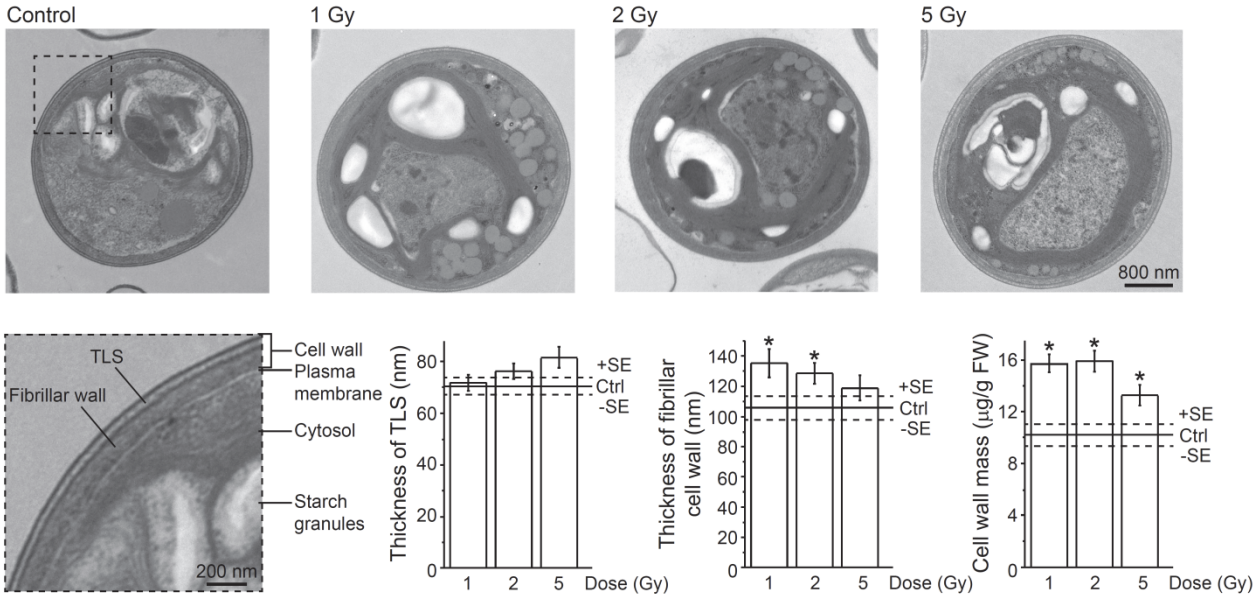

Figure 1. Representative TEM micrographs of *C.sorokiniana* cells and the analysis of cell wall parameters presented in graphs. Full and dashed lines in the graphs represent control (untreated microalgae) values ± standard error. * - statistically significant compare to controls ($p < 0.05$).

It appears that *Chlorella sorokiniana* responds promptly to ionizing radiation by fortifying its 'first line of defence'. The observed changes may be of particular interest for bioremediation, taking into account the capacity of cell wall to bind water-soluble metals, including radionuclides. In addition, cell wall may buffer the influx of reactive oxygen species that are generated in aqueous environment by ionizing radiation.

Acknowledgements
This work has been supported by NATO SPS Programme, Project number G5320.

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