

REVIEW

Of the PhD thesis

Of Tania Toshkova-Yotova

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The presented thesis is written on 315 pages, with 29 figures and 8 tables. It includes Introduction, Materials and Methods, Results and Discussion and References sections with 135 titles 7 of which in Cyrillic.

The Introductions section is a revision of the literature, both recent and classic, on specificity of different alga products and their application in practically all aspects of our life. Despite the growing achievements in the field of experimental and applied algology, only a part of the huge wealth of microalgae species has been studied, and the potential of this useful resource remains unknown and unutilized.

The thesis demonstrates a solid understanding of the state-of-the-art in the research area and the knowledge of the most important and current literature.

The aim is clearly and precisely defined and the tasks are appropriate to this aim.

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The “Materials and Methods” section is very rich it includes 44 methods, most of which interdisciplinary, including:

Algae material;

Cultivation of microalgae, measurements and analyzes;

Gas chromatographic - mass spectral analysis of lipid extract;

Chemical characteristics and composition of extracellular polysaccharide;

Preparation of samples from *Coelastrella* sp. BGV for biological activity test;.

Study of the biological activity of metabolites and extracts of *Coelastrella* sp. BGV;

Investigation of antitumor activity;

MTT test;
Cytomorphological studies using fluorescent methods;
Staining with acridine orange (AO) and ethidium bromide (EtBr);
DAPI staining;
Study of antibacterial and antifungal activities;
Agar diffusion method;
Disc diffusion method;
Antioxidant activity of ethanol extract;
Statistical analysis.

The methodology is described in a way that allows reproduction of the experiments. The rules of good scientific practice are adhered to.

The results are presented clearly, with appropriate controls and statistical analysis. The green algae were cultivated, biomass productivity and specific growth rate estimated. The biochemical composition of the biomass determined. The oil extracts, fatty acids composition and chemical composition of exopolysaccharide content were characterized.

The biological activity of products derived from *C.sp. BGV* has been studied. The antiproliferative activity against HeLa cells and normal mouse fibroblast cells 3T3 was investigated.

Cytomorphological changes of HeLa tumor cells cultured in the presence of algal products were examined using AO, Et.Br. and DAPI. Significant nuclear alterations were found, documented with very good photographs.

Significant antibacterial/anti- different *E. coli* and *St. aureus* strains/ and antifungal /against *C. albicans*/ activities of algal products derived from the green microalga *Coelastrella sp. BGV* were demonstrated.

The antioxidant activity of an ethanol extract from *C. sp. BGV* were inspected and significant activity found.

In the same chapter the results were discussed in relation to the research of others and the thesis demonstrates a good understanding of the implication of the work in a broad scientific context.

I agree with the conclusions made:

1. From the studied four strains of green microalgae of the genus *Coelastrella* - *Coelastrella* sp. BGV, *Coelastrella multistriata* var. *corcontica* (CCALA 308), *Coelastrella multistriata* var. *multistriata* (CCALA 309) and *Coelastrella vacuolata* (CCALA 356), the Bulgarian strain *Coelastrella* sp. BGV showed the highest growth potential in laboratory conditions.

2. The qualitative composition of the biomass of *Coelastrella* sp. BGV in exponential and stationary growth phase is quantitatively balanced, which together with high productivity makes this strain promising for biotechnological use.

3. Eleven fatty acids have been identified in the lipid extract of *Coelastrella* sp. BGV, of which oleic (C18: 1), linoleic (C18: 2) and palmitic (C16: 0) were in the largest amount. The ratio of unsaturated to saturated fatty acids was 3: 1, and that of monounsaturated to polyunsaturated fatty acids was 1: 1.

4. The exopolysaccharide from *Coelastrella* sp. BGV is low molecular weight and contains predominantly neutral sugars. It is composed of seven monosaccharides, two of which have been identified - galactose and fucose

5. Extracellular secretions and components of the biomass of *Coelastrella* sp. BGV showed high biological activity in *in vitro* experiments.

6. The test products obtained from *Coelastrella* sp. BGV, showed varying degrees of antitumor/antiproliferative activity against HeLa human tumor cells (cervical adenocarcinoma) as detected by the MTT assay.

6.1. The highest statistically significant antitumor effect was found for the fatty acids (FAs), followed by the ethanol extract (EE), unsaponifiable substances (US) and exopolysaccharides (EPSH) at both time intervals studied and the effect of FAs (at 24 hours and 48 hours) and US (at 48 hours) was comparable to that of the classic cytostatic Doxorubicin.

6.2. The extracellular secretions in culture medium (CM), low temperature (LT) and high temperature (HT) aqueous extracts induced a statistically significant inhibition of the viability of HeLa tumor cells at both time intervals. The effect of the LT, HT and oil extract (OE) was stronger at the 24th hour, while the effect CM was 2-3 times more pronounced at the 48th hour.

7. Treatment of HeLa tumor cells with extracts and metabolites of *Coelastrrella* sp. BGV induced varying degrees of morphological changes in the cell and nucleus, characteristic of early and/or late apoptosis, established by the application of fluorescent test methods (double staining with AO and EtBr and staining with DAPI).

8. The results of the fluorescent test methods are in accordance with the data obtained from the MTT test and showed that the products isolated from the Bulgarian strain *Coelastrrella* sp. BGVs exert antitumor activity against HeLa tumor cells by induction of apoptosis.

9. Encouraging results have been obtained regarding the therapeutic potential of extracts and metabolites of *Coelastrrella* sp. BGV (ethanol extract, fatty acids, exopolysaccharide and culture medium) against a wide range of Gram-negative and Gram-positive bacteria, as well as fungal pathogen *Candida albicans*. The highest activity was found for the FAs and EE.

10. The ethanol extract from the biomass of *Coelastrrella* sp. BGV has a high content of total phenols, flavonoids and a high level of total antioxidant activity.

11. The obtained results for antitumor, antibacterial and antifungal activities of the extracts and metabolites of *Coelastrrella* sp. BGV show perspectives for future application of this Bulgarian strain in practice.

I also agree with the author's contributions;

1. The biochemical characteristics of the Bulgarian strain of green microalgae *Coelastrrella* sp. BGV were enriched and expanded. The chemical composition of the biomass in two phases of growth - exponential and stationary was characterized. An exopolysaccharide was isolated from *Coelastrrella* sp. BGV for the first time.

2. The fatty acid profile of the oil extract and the chemical composition of the EPSH from *Coelastrrella* sp. BGV were characterized.

3. Original data on biological activity of extracts and metabolites of *Coelastrrella* sp. BGV was obtained.

4. Antitumor activity of extracts and metabolites of *Coelastrrella* sp. BGV against HeLa human tumor cells was established for the first time in *in vitro* experiments.

5. High antitumor activity of fatty acids, unsaponifiable substances, ethanol extract and exopolysaccharides from *Coelastrrella* sp. BGV was found. The activity of fatty acids and unsaponifiable substances against HeLa tumor cells was comparable to that of the antitumor antibiotic Doxorubicin, widely used in clinical practice.

6. Antibacterial activity of fatty acids and ethanol extract of a strain of *Coelastrella* sp. BGV, against Gram-negative pathogens (*E. coli* ATCC, *E. coli* UPEC, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) was found for the first time.

7. It has been found that *Coelastrella* sp. BGV is a rich source of natural antioxidants.

8. The Bulgarian strain of green microalgae *Coelastrella* sp. BGV is a potential source of biomass and products derived from it, for application in the development of new natural drugs for the treatment of tumors and bacterial infections in experimental and clinical conditions.

The candidate's contribution to each aspect of the thesis are clearly stated.

The candidate's contribution to the research and publications, credits and exams is sufficiently large to award her with a PhD, and I strongly recommend it to the scientific jury.

23.11.2020

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