

BABEȘ-BOLYAI UNIVERSITY
FACULTY OF BIOLOGY AND GEOLOGY
DOCTORAL SCHOOL OF INTEGRATIVE BIOLOGY

DOCTORAL THESIS

Summary

Scientific Supervisor

Prof. Dr. *Emeritus* Elena RAKOSY

PhD Student

Maricel BOCĂNEALĂ

CLUJ-NAPOCA

2025

BABEȘ-BOLYAI UNIVERSITY
FACULTY OF BIOLOGY AND GEOLOGY
DOCTORAL SCHOOL OF INTEGRATIVE BIOLOGY

DOCTORAL THESIS

Summary

**Advancing cyanobacterial value-added products through a
multidisciplinary approach, integrating scientific insight with
industrial relevance**

Scientific Supervisor

Prof. Dr. *Emeritus* Elena RAKOSY

PhD Student

Maricel BOCĂNEALĂ

CLUJ-NAPOCA
2025

Table of Contents

Outline of the thesis	1
Preface	4
CHAPTER I	5
General introduction	5
I.1. Introduction	5
I.2. Cyanobacteria usage and products	6
I.2.1. Pigments	7
I.2.1.1 Chlorophyll	7
I.2.1.2. Carotenoids	8
I.2.1.3. Phycobiliproteins	9
I.2.1.4. Scytonemin	10
I.2.2. Biofuels	10
I.2.2.1. Biomass processing	11
I.2.2.2. Hydrogen production	12
I.2.3. Bioplastics	12
I.2.3. Bioactive compounds	13
I.2.4. Exopolysaccharides	14
I.2.5. Recent advancements for obtaining cyanobacterial products	15
I.2.5.1. Strain engineering	15
I.2.5.2. Photobioreactor innovation	16
I.3. Exopolysaccharides produced by cyanobacteria	17
I.3.1. Structural diversity of exopolysaccharides	18
I.3.1.1. Monosaccharide C5 and C6	18
I.3.1.2. Heteropolymers	18
I.3.1.3. Varied physical properties	19
I.3.2. Role of exopolysaccharides in ecology and physiology	19
I.3.2.1. Biofilm matrix and adhesion	20
I.3.2.2. Protection and stress tolerance	20
I.3.2.3. Motility and development	21
I.3.2.4. Nutrient reservoirs	22
I.3.3. Applications of cyanobacterial exopolysaccharides	22

I.3.3.1. Medical and pharmaceuticals applications.....	22
I.3.3.2. Environmental applications.....	23
I.3.3.3. Food and industrial applications.....	24
I.4. Proposed mechanism of exopolysaccharides production.....	24
I.4.1. Biosynthetic pathways and gene clusters	24
I.4.2. Regulation of exopolysaccharide synthesis under environmental conditions	27
I.4.3. Enhancements through genetic engineering	29
I.5. Biotechnological processes for exopolysaccharides extraction and purification.....	30
I.6. Aims of the thesis.....	32
I.7. References.....	33
CHAPTER II.....	43
Charting the academic output on cyanobacterial exopolysaccharides and their industrial applications.....	43
II.1. Introduction.....	43
II.2. Materials and methods.....	44
II.2.1. Bibliometric data retrieval	44
II.2.1.1. Articles selection criteria for the filtered dataset.....	45
II.2.2. Filtered dataset	45
II.2.3. Espacenet patent data	46
II.3. Results.....	46
II.3.1. Charting the research output on cyanobacterial exopolysaccharides - full dataset	46
II.3.1.1. Annual scientific production.....	47
II.3.1.2. Authorship and research networks.....	49
II.3.1.3. Publishing avenues for cyanobacterial exopolysaccharides research.....	51
II.3.1.4. Research interests.....	52
II.3.2. Charting the research output on cyanobacterial exopolysaccharides industry applications - filtered dataset	57
II.3.3. Espacenet patent analysis	61
II.4. Discussion.....	65
II.5. Conclusions.....	67
II.6. Funding.....	67
II.7. Acknowledgements.....	67
II.8. References.....	68

CHAPTER III.....	74
Spectroscopic and chromatographic characterization of in house purified exopolysaccharides from <i>Coelomoron pussilum</i> AICB1012 species.....	74
III.1. Introduction.....	74
III.2. Materials and methods.....	78
III.2.1. Materials.....	78
III.2.2. Production, purification and characterization of exopolysaccharides samples.....	78
III.2.3. FT-IR analysis.....	79
III.2.4. HPLC analysis.....	79
III.2.5. SERS spectroscopy measurements.....	79
III.3. Results and discussion.....	80
III.3.1. Spectroscopic characterization.....	80
III.3.1.1. SERS investigation.....	80
III.3.2. FT-IR analysis.....	82
III.3.3. Chromatographic analysis.....	84
III.4. Conclusions.....	85
III.5. Funding.....	87
III.6. Acknowledgements.....	87
III.7. References.....	87
Chapter IV.....	93
Profiling the Spirulina dietary supplements available on the Romanian market.....	93
IV.1. Introduction.....	93
IV.2. Materials and methods.....	95
IV.2.1. Data set.....	95
IV.2.2. Sample preparation and elemental analysis.....	96
IV.2.3. Isotope ratio mass spectrometry analysis.....	97
IV.2.4. Data analysis.....	97
IV.3. Results and discussion.....	99
IV.3.1. Elemental results.....	99
IV.3.2. Isotopic results.....	99
IV.3.3. Analysis of Spirulina profile.....	100
IV.4. Conclusions.....	111

IV.5. Funding.....	112
IV.6. Acknowledgments.....	112
IV.7. References.....	112
CHAPTER V.....	118
General conclusion and perspective.....	118
APPENDICES CHAPTER II.....	121
APPENDICES CHAPTER III.....	154
APPENDICES CHAPTER IV.....	157
LIST OF PUBLICATIONS INCLUDED IN THE THESIS AS CHAPTERS.....	158
LIST OF PUBLICATIONS NOT INCLUDED IN THE THESIS.....	158
CONFERENCES ATTENDANCES.....	159
INVOLVMENT IN RESEARCH PROJECTS AS TEAM MEMBER.....	159
ACKNOWLEDGEMENTS.....	161

Outline of the thesis

Cyanobacteria are an ancient lineage of organisms with profound biogeochemical impact throughout the Earth's history. Their most notable achievement being the Great Oxidation Event, a massive side effect to the evolution of the photosynthetic apparatus that brought the atmospheric oxygen level to the levels of today and shaped the world as we know it. This ancient lineage is not a stranger to the human societies who have used members from this group for food, feed, folk remedies or cosmetics. These organisms become more and more valuable in humanity's toolkit, especially with the discoveries of the extracellular polysaccharides (EPS) produced by them.

The work presented in this thesis aims to:

- I. to analyze the academic output on cyanobacterial exopolysaccharides and their industrial applications;
- II. to characterize exopolysaccharides using combined spectroscopic and chromatographic methods, to enable enhanced and diverse output for the industrial sector;
- III. to explore nutrient and toxic element profiles in *Spirulina* products available on the Romanian market.

Summary

CHAPTER I. General introduction

Chapter I presents the general knowledge on cyanobacterial products, including foods, pigments, bioactive compounds, biofuels and bioplastics. Special attention is given to exopolysaccharides, detailing their structural features, known and potential roles for cyanobacterial physiology, and their potentials biotechnological applications. This chapter highlight fundamental insights and practical advantages for exploitation of cyanobacterial products.

The cyanobacterial biomass was recognized as a valuable resource throughout the history of human civilization. The oldest historical record seems to be the conquistadores' journals, that describe tecuitlatl (Farrar, 1966).

Cyanobacteria are a group of organisms able to perform oxygenic photosynthesis. Their ability to photosynthesize is based on 3 major classes of colorful compounds that can interact with light, namely chlorophyll, carotenoids and phycobiliproteins. These bioactive compounds can be used for diverse biotechnological application in cosmetic, food, or pharmaceutical industries (Assunção et al., 2022).

Chlorophyll (colored, conjugated polyene) is the most abundant photosynthetic pigment in cyanobacteria and other photoautotrophic organisms. Carotenoids are non-polar pigments that play a crucial role in photoprotection. They can absorb light in the visible spectrum that differs from the absorption spectrum of chlorophyll molecules. Phycobiliproteins are water-soluble, florescent compounds, with the main biological role of absorbing light in the visible spectral range and protecting cyanobacterial cells when exposed to high intensity of light.

Cyanobacteria have a physiologically feature that sets them apart from other Gram-negative bacteria, namely their mucilaginous external layers (called slime, mucilage, sheath, capsule, or glycocalyx). Chemically speaking, this layer is formed most of the times from polysaccharides. These polysaccharides can be categorized based on their relation with the bacterial cell: sheath, capsule or slime, and soluble polysaccharides.

Polysaccharides are an economically advantageous biological molecule produced by cyanobacteria due to their versatility in industrial application. For economic feasibility, industrial scale production uses mostly microbial sources of such polymers. The interest in cyanobacteria as a source for exopolysaccharides comes from their photosynthetic features (higher ability to sequester carbon compared to plants, releasing oxygen), their nutrient requirements are quite basic

and can also be covered with minimal additions by different wastewater sources, the ability to produce diverse polymeric products with diverse applications with some strains being able to also produce them in high volumes. Adding on these advantages the fact that these polymeric products are biodegradable their economic appeal grows even further.

Cyanobacterial exopolysaccharides are made up of repeating unit, that are themselves formed by different monosaccharides. A repeating unit is made up of between five and eight monosaccharides, but extremely complex cases have been reported where 15 monosaccharides were present in a repeating unit. These varied physico-chemical characteristics are directly result from the structural diversity of cyanobacterial exopolysaccharides and are intricately linked to their environmental role as well as their potential biotechnological applications.

The biosynthesis process can be divided into three steps, across different cellular compartments. Firstly, in the cytoplasm, takes place the activation of the monosaccharides and conversion into sugar nucleotides. Secondly, at the plasma membrane, the activated sugars are assembled into repeating units by sequential additions onto a lipid carrier. Lastly, still at the plasma membrane, on the periplasmic face, the polymerization of the repeating units takes place. This last step is also where the new polymers are exported at the cell surface (Pereira et al., 2009, Potnis et al., 2021).

For success with biotechnological production and application of cyanobacterial exopolysaccharides culture parameter identification is paramount. To date, a high volume of research is produced that looks for the most appropriate culture condition and nutritional medium that would result in obtaining qualitative exopolysaccharides in profitable amounts.

The potential of exopolysaccharides in industrial applications can also be exploited through genetic engineering. Even though not all the details regarding the biosynthesis pathways are extensively investigated, the opportunities for genetic manipulation for amending the quantity or quality of polymers produces by cyanobacterial strains are present in every step of the biosynthesis pathway.

The methods selected for the isolation and purification of exopolysaccharides can influence the final yield and quality, with some protocols needing optimization to align the obtained product with the actual use. On top of this, the suitability of these protocols also needs to take into account ease of use, effectiveness and scalability.

Summary

CHAPTER II. Charting the academic output on cyanobacterial exopolysaccharides and their industrial applications

Chapter II was accepted for publication as **Bocăneală, M.**, Goron, G. M., Rakosy-Tican, E. 2025.

Charting the Academic Output on Cyanobacterial Exopolysaccharides and Their Industrial Applications. Studia Universitatis Babeş-Bolyai, Biologia,

Cyanobacterial EPS have demonstrated potential in various industrial settings. In the food industry, they can serve as natural thickeners, stabilizers, and emulsifiers, improving the texture and shelf-life of products (Vicente-García et al., 2004; Jindal et al., 2013; Najdenski et al., 2013; Nath et al., 2021). In pharmaceuticals, their bioactive properties, such as antioxidant, antibacterial, and antiviral activities, make them suitable for developing new therapeutic agents (Gacheva et al., 2013; Bhatnagar et al., 2014; Shen et al., 2018; Flores et al., 2019; Ramachandran et al., 2020). Environmental applications include bioremediation, where EPS can bind heavy metals and pollutants, facilitating their removal from contaminated sites (Colica et al., 2010; Santos et al., 2014; Mota et al., 2016; Mohamed et al., 2023). Additionally, in agriculture, EPS-producing cyanobacteria contribute to soil stabilization and fertility, promoting sustainable farming practices (Van Camp et al., 2022; Falsini et al., 2023; Vinoth et al., 2023).

Our study offers an analysis of the academic and industrial landscape surrounding cyanobacterial EPS between 2004 and 2023. By using bibliometric and patent analyses, we have identified key trends, contributors, and an increased potential for industrial applications of cyanobacterial EPS research. Below, we discuss the implications of these findings, their relevance to the scientific and industrial communities, and the limitations of this study.

Our findings reveal a steadily growing body of research on cyanobacterial EPS, with an annual growth rate of 13.14% over the study period (2004–2023). This growth shows an increased interest in this field. However, the thematic focus remains concentrated around healthcare, pharmaceuticals, and environmental applications, hence we understand that these industries dominate the research agenda.

The analysis of authorship and collaborative networks highlights the concentrated effort among a select group of researchers and institutions, particularly in China, the USA, India, and

Europe. This collaboration aligns with the multidisciplinary nature of EPS applications but also indicates geographic and institutional disparities in research intensity and focus.

The filtered dataset illustrates a notable interest in the healthcare and environmental sectors, accounting for over half of the research focus. The bioactive and bioremediation properties of EPS have driven these trends, making them promising candidates for drug development, wound healing, and pollutant removal. Despite this focus, research on applications in the food, agriculture, and energy sectors remains sporadic and underdeveloped, possibly due to limited funding.

The positive annual growth rate of publications, dominance of leading global economies as top research producers, and the applied focus of top journals and highly cited articles highlight the field's translational orientation. These findings point to the fact that cyanobacterial EPS research is oriented towards potential applications.

The increase in patent filings post-2014 mirrors the expansion of potential industrial interest, with the USA and China being prominent players. However, a disconnect between academic output and patent filings is evident, particularly in countries like China, which leads in publication frequency but lags in patent activity. This may suggest challenges in translating academic research into commercial products.

We note potential IP-related search limitations – the Espacenet search was done based on two terms: cyanobacteria and exopolysaccharide. It is possible that some patent filings would be more specific and name a particular cyanobacteria genus, species, strain, a particular extracellular polysaccharide, or process, and not necessarily use these relatively general terms. However, we have conducted the search using the 'full text' filter, meaning all patents having the keywords within the documentation were included. While this does not guarantee all relevant patents were included, it brings the result closer to reality.

Regarding the publishing data collection – our study relies on Web of Science for bibliometric data, and we have included only English-language articles. We have used a relatively general search string, which should have included the majority of relevant research. We did not include in the search query specific cyanobacteria species names, but we used the 'all fields' option. Since it is unlikely that papers focusing on specific cyanobacterial strains would not have used the term cyanobacteria or similar in any of the indexing fields, we believe all relevant results have been included.

Summary

CHAPTER III. Spectroscopic and chromatographic characterization of in house purified exopolysaccharides from *Coelomorion pussilum* AICB1012 species

Manuscript under review

Cyanobacteria are a group of organisms that are growing in popularity within the industrial sector (Liu et al., 2021; Diaz et al., 2023; Zedler et al., 2023). Their abilities to capture CO₂, their diverse enzymatic apparatus, and their ability to produce varied industrially useful compounds are some of their most valued assets. *Coelomorion pussilum* AICB1012 is such a species, but with scarce studies on its spectral or biochemical particularities (Komárek, 1989). Previous studies have shown that this strain of cyanobacteria can be a source of phycoerythrin, phycocyanin, and allophycocyanin (Porav et al., 2020). Such products are obtained from processing the biomass. In order to better exploit the growth process of these cyanobacteria, the exhausted culture media was also used for extraction of potentially valuable compounds. The routinely found compounds in cyanobacterial exhausted culture media are released exopolysaccharides, a class of diverse compounds with varying biological activities (Wu et al., 2021; Liang et al., 2024).

FT-IR Spectroscopy has been used for functional groups analysis that are present in the EPS samples. Raman spectroscopy is commonly used to identify bands associated with vibrational modes of functional groups within a solid or liquid sample and to discriminate between slight spectral fluctuations in multi-component analytes. Qualitative information about the monosaccharide composition was obtained by comparing peak retention times with standard sugars.

Coelomorion pussilum AICB1012 was grown in BG11 media with modification (Allen and Stanier, 1968). All cultures were maintained at 25°C, under continuous lighting conditions, and the optimal mixing was maintained by continuous air bubbling. The cultures were exposed to different C:N percentage ratios as follows: 100:100 (control BG11), 100:50, 50:100, 50:50, 50:10, 10:50.

Considering the similar Raman spectral features for all samples, it is a matter of band positions and their relative intensities that is in our focus for discriminating the sugar components. The most intense bands are those ascertained to the mannose, galactose, rhamnose and glucose monosaccharides, as also revealed by the additional vibrational and chromatographic analyses. With minor contributions we identified also the specific bands for fucose, and glucuronic acid such as 587 cm⁻¹, 938-943 cm⁻¹ and 1373 cm⁻¹ (Park and Kim, 2010). The presence of Raman bands specific for

carboxylate might reveal the formation of uronic acids via oxidation of the hydroxyl group to a carboxylic acid (Park and Kim, 2010).

The FT-IR spectra of lyophilized EPS samples show similar pattern, with small differences in band intensities. As previously reported (Hong et al., 2021), the spectra of investigated polysaccharides are divided into five spectral ranges. Region I ($4000\text{--}2500\text{ cm}^{-1}$) is dominated by a broad and intense band from $3000\text{--}3600\text{ cm}^{-1}$ corresponding to the stretching vibrations of hydroxyl function. The stretching vibrations of CH and CH_2 groups are appearing in this region at 2851 cm^{-1} and 2922 cm^{-1} . The second and third region ($1800\text{--}1500\text{ cm}^{-1}$ and $1500\text{--}1200\text{ cm}^{-1}$) is generally assigned to stretching vibrations of double bonds and vibrations of groups with local symmetry. In our particular case, the large band between $1507\text{--}1423\text{ cm}^{-1}$ is more likely to represent the in-plane bending of C-H bonds, while the 1645 cm^{-1} is due to vibration of water molecules (Wiercigroch et al., 2017). The region between $1200\text{--}800\text{ cm}^{-1}$ represents the ‘fingerprint’ area and, in case of carbohydrates, it gives some information on the configuration of the glycosidic bond. The absorptions at 1097 cm^{-1} and 1059 cm^{-1} are attributed to the stretching vibrations of pyranose ring and the one at 866 cm^{-1} is indicative for the β -configuration of the sugar units (Edelmann et al., 2001). The vibrations in the region under 800 cm^{-1} are attributed to the skeletal modes of the carbohydrate rings.

The HPLC analysis of the hydrolyzed samples was employed to qualitatively assess the constituent sugar residues of the EPS. Except for the EPS 100:50 sample, the uronic acids were identified in all the samples, with different ratios (retention times 8.5 and 9.1 minutes). The presence of glucose (9.7 minutes) can be clearly stated only for the EPS 100:50 sample. The EPS 10:50, 50:10, 50:100 and 100:50 samples have mannose or galactose (retention time 10.3 minutes). Fructose and rhamnose were identified in EPS 50:100 and EPS 100:50 samples. The chromatograms of the EPS 50:10 and EPS 50:50 samples show also the presence of some amount of fucose.

Overall, the results presented in this work show that spectroscopic and chromatographic techniques can be efficiently employed in order to assess the molecular characterization of a bioproduct with minimal effort and resources. Cyanobacterial exopolysaccharides from exhausted media are not only a value-added product, but can also be exploited in accordance to the principles of circular economy. Rare sugar content translates to market opportunities, as they are not easily found in nature. Their presence in the structure of polysaccharide means physical and chemical characteristics that determine unique biological activities (emulsion stabilizers, thickeners, materials for nano/microparticles, or scaffolds for tissue engineering to name a few) (Roca et al., 2015).

Summary

Chapter IV. Profiling the Spirulina dietary supplements available on the Romanian market

Chapter IV was published as **Bocăneală, M.**, Hategan, A.R., David, M., Dehelean, A., Cristea, G., Szűcs-Balázs, J.Z., Rakosy-Tican, E. and Magdas, D.A., 2025. Profiling the Spirulina Dietary Supplements Available on the Romanian Market. *Applied Sciences*, 15(5), p.2658.

doi: [10.3390/app15052658](https://doi.org/10.3390/app15052658)

Over the last two decades, food supplement consumption has grown around the world. In addition, the market interest for these products increased during the COVID-19 pandemic, as a boost for the immune system (Djaoudene et al., 2023). In this context, one of the most consumed dietary supplements, worldwide, is Spirulina. *Arthrospira platensis*, commercially known as Spirulina, is filamentous cyanobacteria able to cope with high alkalinity, high temperature, and high salinity (Cepoi and Zinicovscaia, 2020). Spirulina is highly valued as a dietary supplement, feed, and a cosmetic agent. It has high protein content (up to 70%), high free fatty acid content (up to 80% of total lipids), and relatively low carbohydrate content (up to 20%). It is also a good source of vitamins (A, B1, B2, B3, cyanocobalamin, E, etc.) (Mishra et al., 2014) and minerals (iron, calcium, and magnesium). Moreover, Spirulina has a long list of bioactive compounds, from chlorophyll, beta-carotenes, and xanthophyll to polysaccharides to peptides and proteins, the best known being the blue pigment phycocyanin, with the physiological function of a light-harvesting antenna and a powerful antioxidant in animal organisms (Romay et al., 2003). As a result, multiple studies have reported antioxidant, antitumor, anti-inflammatory, immunostimulant, and hypoglycemic activities (Ismail et al., 2009; Deng and Chew, 2010; Nasirian et al., 2018).

The present work comprises the first comprehensive analysis of all types of Spirulina samples available on the Romanian market, aiming to shed light on the diversity of Spirulina products from a macro-, micro-, and ultramicro-element perspective in order to assess if this product, treated by some as a superfood, can actually deliver on its promises. Moreover, statistical analysis was applied in order to discuss the differences in the macro- and micro-elemental content and the marketing influences.

A data set consisting of 66 Spirulina samples, available on the online market in Romania and commercialized in the form of tablets, powder and capsules, having several packaging was used in the frame of the current study. Among these samples, 49 samples had a labeled geographical origin

(non-EU—45; EU—4), while 32 samples were specified as being produced under an organic growing regime. Additionally, one in-house *Spirulina* sample was analyzed.

The quantification of the elements was performed by using a flame atomic absorption spectrometer (FAAS). To record the ^{13}C isotopic signature of *Spirulina*, isotope ratio mass spectrometry (IRMS) was used. The statistical analysis of the experimental data, encompassing the ^{13}C isotopic value and 23 elemental determinations of the *Spirulina* samples, was conducted based on Python programming. PCA, an unsupervised multivariate statistical technique relying on the eigenanalysis of the covariance matrix, was applied in the frame of the present study as an exploratory data analysis method that allows for the identification of potential groupings of similar samples, as well as the presence of outliers or isolated points. Subsequently, the analysis of variance (ANOVA) was applied in order to identify the isotope or elemental determinations that might be reflective of the type of *Spirulina* formulation (i.e., capsule, tablets, or powder), the package type and material, the geographical provenance, and the specified growing regime.

Overall, the most abundant mineral found in *Spirulina* samples was K, with the exception of three samples that displayed Na at the highest concentration, while the lowest concentrations were seen for Cd and Sb. The isotopic fingerprint of the investigated *Spirulina* samples ranged between -33.6 and -16.0‰ , with a mean value of -23.9‰ . These values are consistent with those published by Kejžar et al. [23] for *Spirulina* spp., recording an average value of $\delta^{13}\text{C} = -23.0\text{‰}$. The ^{13}C isotopic signature of the studied *Spirulina* samples offers information regarding their carbon source.

Our study analyses all the available types of *Spirulina* samples commercialized on the Romanian market, with one grown in-house. The results presented are in agreement with what is generally known, that is, that *Spirulina* is a good source of macro- and micro- nutrients such as Ca, Mg, Fe, and Zn. As for other potentially harmful elements, such as As, Pb, and Cd, while the values are below regulated thresholds, they still need to be carefully taken into account when *Spirulina* is taken in prolonged courses of treatment. Generally, not all labels specify a comprehensive mineral composition. Given that our study revealed significant variability in the elemental concentrations of the samples and considering the absence of strict regulations for this dietary supplement, consumer risk is amplified. Regarding the influence of the formulation, packaging type, and geographical origin, our study did not reveal any significant variations among classes. Moreover, no significant differences have emerged from our analyses between organic-labeled and non-labeled samples, a fact that emphasized that the 'eco/bio'-labeled cultivation regime is primarily motivated by economic interests, without a justification from a nutritional point of view.

Summary

CHAPTER V. General conclusion and perspective

The impact of Cyanobacteria as a group of organisms can be measured on geological time and space scale. Their ancient history allowed them not only to colonize new environmental niches, but also to change these niches, making them more habitable for themselves and other organisms. They use the sunlight for energy and are involved in the biogeochemical cycles of most vital elements for life on earth. The attention they garnered as potential parts for solution to the majority of humanity's problems is not an exaggeration if their diversity and versatility is taken into account. However, important efforts need to still be made so that these organisms to achieve their full potential.

Taking this into consideration, this thesis aims to advance the development of cyanobacterial value-added products through multidisciplinary means, integrating literature and patent analysis, molecular characterization and applied screening of commercial products. These outcomes of this work are reflected as follows:

- I. the analysis of academic literature and patent data revealed a growing interest in cyanobacterial exopolysaccharide, with increasing emphasis on their functional properties and industrial applications;
- II. the characterization of exopolysaccharide through combined spectroscopic and chromatographic methods led to the identification of rare sugars and demonstrated that these polymers can serve as functional added-value byproducts;
- III. the element analysis of Spirulina product available on the Romanian market highlighted their composition in term of macro- and microelements (e.g. Ca, Mg, Fe, Zn), while also providing insights into the presence of potentially harmful elements (e.g. Pb, As, Cd).

The originality of this work lies in presenting the first bibliometric analysis of scientific literature and patent data related to the industrial potential of cyanobacterial exopolysaccharides, the first investigation of *Coelomorion pussilum* AICB 1012 with a focus on released exopolysaccharides, and the first comprehensive evaluation of Spirulina dietary supplements available on the Romanian market.

As future perspective, the full exploration of *Coelomorion pusilum* AICB 1012 potential for biotechnological application would be the next big endeavor. The full optimization of the optimal

culture conditions for high yield biomass should be the first step. A biorefinery approach for a two-step cultivation process would be the surefire way to extract the full spectrum of bioactive compounds from the biomass. Of course, in achieving the full exploitation of this cyanobacterial strain transcriptomics, proteomics and metabolomics could also be employed that would not only support the success of this venture but also provide valuable information about biological pathways and processes.

LIST OF PUBLICATIONS INCLUDED IN THE THESIS AS CHAPTERS

Chapter II

Bocăneală, M., Goron, G. M., Rakosy-Tican, E. 2025. Charting the Academic Output on Cyanobacterial Exopolysaccharides and Their Industrial Applications. *Studia Universitatis Babeş-Bolyai, Biologia, Manuscript accepted. (BDI)*

Chapter III

Bocăneală, M., Brezeştean, I., Rakosy-Tican, E. and Dina, N.E., 2025. Spectroscopic and chromatographic analysis of in house purified exopolysaccharides from *Coelomorion pussilum* AICB1012 species. *Manuscript under revision*

Chapter IV

Bocăneală, M., Hategan, A.R., David, M., Dehelean, A., Cristea, G., Szücs-Balázs, J.Z., Rakosy-Tican, E. and Magdas, D.A., 2025. Profiling the Spirulina Dietary Supplements Available on the Romanian Market. *Applied Sciences*, **15**(5), p.2658. <https://doi.org/10.3390/app15052658> (ISI IF₂₀₂₃ = 2.5, AIS₂₀₂₃ = 0.428)

LIST OF PUBLICATIONS NOT INCLUDED IN THE THESIS

1. Porav, A.S., **Bocăneală, M.**, Fălămaş, A., Bogdan, D.F., Barbu-Tudoran, L., Hegeduş, A. and Dragoş, N., 2020. Sequential aqueous two-phase system for simultaneous purification of cyanobacterial phycobiliproteins. *Bioresource technology*, 315, p.123794. <https://doi.org/10.1016/j.biortech.2020.123794> (ISI IF₂₀₂₀ = 9.642, AIS₂₀₂₀ = 1.245)
2. Brezeştean, I., **Bocăneală, M.**, Gherman, A.M.R., Porav, S.A., Kacsó, I., Rakosy-Tican, E. and Dina, N.E., 2021. Spectroscopic investigation of exopolysaccharides purified from *Arthrospira platensis* cultures as potential bioresources. *Journal of Molecular Structure*, 1246, p.131228. <https://doi.org/10.1016/j.molstruc.2021.131228> (ISI IF₂₀₂₁ = 3.841, AIS₂₀₂₁ = 0.315)
3. Ciorîţă, A., Suci, M., Rostas, A.M., Tarţa, A., Popovici, G., **Bocăneală, M.**, Nekvapil, F., Macavei, S.G., Potara, M., Marica, I. and Kacsó, I., 2024. Interaction of Low-Density Polyethylene Nanofragments with Autotrophic and Chemotrophic Bacteria. *ACS Sustainable Chemistry & Engineering*, 12(29), pp.10831-10840. <https://doi.org/10.1021/acssuschemeng.4c02440> (ISI IF₂₀₂₃ = 7.1, AIS₂₀₂₃ = 1.358)

CONFERENCES ATTENDANCES

1. Porav, A.S., **Bocăneală, M.**, Falamas, A., Barbu, L. and Dragos., N., (2019) A simple method for obtaining high purity phycobiliproteins
 - Poster presentation — 12th International Conference Processes in Isotopes and Molecules, September 25-27, 2019, Cluj-Napoca, Romania;
2. Brezeştean, I., **Bocăneală, M.**, Gherman, A.M.R., Porav, A.S., Kacsó, I., Rakosy-Tican, E., Dina, E.N., (2021) Spectroscopic investigation of exopolysaccharides purified from *Arthrospira plantensis* cultures as potential bioresources
 - Poster presentation — 7th Edition of BIO.T.A. Symposium, November, 19-20, 2021, Cluj-Napoca, Romania;

INVOLVMENT IN RESEARCH PROJECTS AS TEAM MEMBER

- **2018 - 2020** — Complex projects completed in CDI consortia PN-IIIP1-1.2-PCCDI2017-0010; title: ‘Emerging molecular technologies based on micro and nano-structured systems with biomedical applications TEHNOBIOMED’; project director: Dr. Ioan Turcu
 - National patent application OSIM no. A00882 11/12/2019;
Dorobanţu, I., Neagu, L., Porav, S., Fălămaş A., **Bocăneală M.**;
Procedure for separating specific antibodies from polyclonal anti-phycoerythrin antisera using an affinity column with the SiO₂-ovalbumin-phycoerythrin nanoimmunosorbent. (Procedeu de separare a anticorpilor specifici din antiseruri policlonale antificocianină pe coloană de afinitate utilizând nanoimunosorbentul SiO₂-ovalbumină-ficocianină)
 - National patent application OSIM no. 774/25/03/2021;
Coman, C., Onu, A., Codiţă, I., Dinu, A.I., Dorobanţu, I., Neagu L., Stoica R.D., Mustăciosu C.C., Porav S., **Bocăneală M.**;
Improved procedure for obtaining polyclonal hyperimmune antisera against phycoerythrin and phycocyanin (Procedeu îmbunătăţit de obţinere a unor antiseruri hiperimune policlonale antificoeritrină şi antificocianină)

- **2021 – 2023** — Projects for exploratory research PN-III-P4-ID-PCE-2020-1463; title: ‘Unveiling the mystery behind the strong polydopamine adhesion: an original approach by introducing local isotopic markers IZO-PDA’; project director: Dr. Claudiu Filip
- **2024 – 2026** — The National Recovery and Resilience Plan Program PNRR-III-C9-2022-I8; title: ‘Development of New Polymeric Antibacterial Materials’; principal investigator: Dr. Anzar Khan

