**INTERDISCIPLINARY DOCTORAL SCHOOL**

**Faculty: Materials Science and Engineering**

**Eng. Ioana GHIUȚĂ (căs. Popescu)**

# CERCETĂRI PRIVIND BIOSINTEZA NANOPARTICULELOR METALICE UTILIZÂND MICROORGANISME

# RESEARCHES ON THE BIOSYNTHESIS OF METAL NANOPARTICLES USING MICROORGANISMS

REZUMAT / ABSTRACT

Scientific coordinator:

Prof. dr. eng. Daniel MUNTEANU

BRAȘOV, 2018

D-lui (D-nei) .....

**STRUCTURE**

**The Doctoral Commission**

Named by the Order of the Rector of TransIlvania University of Brasov
Nr. 9015 din 14.12.2017

| | |
|---|---|
| PRESIDENT: | Prof. dr. eng. Pisu Teodor MACHEDON<br>Dean of the Faculty of Materials Science and Engineering<br>Transilvania University of Brasov |
| SCIENTIFIC COORDINATOR: | Prof. dr. eng. Daniel MUNTEANU<br>Transilvania University of Brasov |
| REVIEWERS: | Prof. dr. eng. Andreas ANAYIOTOS<br>Cyprus University of Technology, Cipru |
| | Prof. dr. eng Joseph KOST<br>Ben-Gurion University of the Negev, Israel |
| | Prof. dr. eng. Mircea Horia ȚIEREAN<br>Transilvania University of Brasov |

Date, time and place of public speech for the PhD thesis: 19 January 2018, 10:00, room UII3, Sergiu Chiriacescu Aula.

Possible appraisals or comments on the content of the paper will be sent electronically, in a timely manner, to the address ioana.ghiuta@unitbv.ro

At the same time, we invite you to take part in the public hearing to support the PhD thesis.

Thank you.

# CONTENT

## Acknowledgements

After an intense period of scientific activity in doctoral studies, I want to take sometime to express my recognition to some wonderful people who have helped me reach the end of this research, which is often only the beginning of an important stage.

First of all, I feel honored to express my gratitude to **Prof. dr. eng. Daniel Munteanu**, who, as a scientific adviser, led me to develop a critical and creative thinking specific to the contemporary researcher. The support he has provided over the years of study has strengthened my determination since their beginning, since when I considered it to be a magistral teacher for conducting my doctoral paper.

In this way, I wish to thank the coordinators, whom I was fortunate to be guided during the two mobility exercises during the doctoral studies:

- **Prof. dr. eng. Andreas Anayiotos**, of Cyprusl University of Technology, Limassol, for his trust and support. I would like to thank him for a balanced development based on prioritization.
- **Asst. prof. dr.Ioannis Vyrides** of the Cyprusl University of Technology, Limassol, for the window of opportunity to juggle with the fascinating elements of nanotechnology. I also want to thank him for the integrated optimism during the collaboration.
- **Prof. dr. eng. Joseph Kost**, of Ben-Gurion University, Negev, Beersheva, Israel, for support, for the professional character and the progressive development of the ability to work within a team.

I would like to thank eminently **Dr. eng. Rodica Wenkert** of the Soroka University Hospital in Beersheva, Israel, for the elements implemented in the nuance of the work, for the altruistic advice and humorism applied to scientific research.

I would like especially to thank **Dr. eng. Strul Moisa** for moral guidance and unswerving support. Thanks for the valuable lessons he had offered, who have influenced my choices in a positive way through perseverance and a great way to deliver these lessons.

I would like to thank the group of the Department of Materials Science, Transilvania University, for the support, understanding and guidance given during the studies. I am particularly grateful to the steering committee, **Prof. dr. eng. Aurel Crişan**, **Prof. dr. eng. Ioan Milosan**, **Prof. dr. eng. Bela Varg**a, who represented the perfect team for the names that is representing. I would like to thank them for the advice offered with patience and wisdom. I also thank Mr **Lector Daniel Cristea**, PhD, for the continuous guidance and counseling provided in most of the stages of the research. I especially appreciate the collaboration and interactions we have had. Also, I would like to **thank Prof. dr. eng. Pisu Teodor Machedon** and **Prof. dr. eng. Mircea Țierean** for their support and involvement in the public submission of the thesis.

I would especially like to thank **Dr. Arevik Vardanyan**, for her encouragement, patience, insightful advice, and constructive feedback during the entire period of research work.

In particular, I would like to express my appreciation to my husband for the patience and sustained support he has provided in this paper. I thank the family for the education they offered and the support for its development.

## NOTATION LIST

- Ag NPs – silver nanoparticles
- AgCl NPs – silver chloride nanoparticles
- ZnO NPs - zinc oxide nanoparticles
- B.s. - *Bacillus subtillis*
- B.a. - *Bacillus amyloliquefaciens*
- R.m. - *Rhodotorulla mucilaginosa*
- P. – *Pantoea*
- R.p. – *Raoultella Planticola*
- E.f. –*Enterococcus faecalis*
- Top-down - Top-down method
- Bottom-up – Bottom-up method

## ABBREVATION LIST

- ICP-MS – Inductive Coupled Plasma, Mass Spectroscopy
- UV—vis – Ultra-violet and visible spectroscopy
- SEM – Scanning electron microscopy scanning / electronic scanning microscope
- TEM - Transmission electron microscopy / electronic transmission microscope analysis
- AFM - Atomic force / atomic force microscopy analysis
- DLS – DLS – Dynamic Diffusion of Light
- SAED – Electron diffraction in selected area
- XRD –X-Ray diffraction
- FTIR – Fourier transform infrared spectroscopy
- RAMAN – Raman Spectroscopy
- EDSX – Analysis by X-ray Dispersion Spectroscopyv
- NADPH – Nicotinamide adenine Nicotinamide Hydrogenphosphate
- NADP – Nicotinamide Adenine Dinucleotide Phosphate

# INTRODUCTION

## 1. Introduction into the theme of the research thesis

The term nanotechnology was first used in the scientific field in 1974. At that time, Professor Norio Taniguchi defined nanotechnology as a production technology with a very high accuracy and ultra-fine dimensions, namely, precision and fineness being of the nanometer order [208]. However, the first mentions of Nano-scale technological processes, deliberately created and applied, but later referred to as nanotechnology, are found in Richard Feynman's well-known reading "There's a Plenty of Room at the Bottom". On December 29, 1959 at the American Physical Society at the California Institute of Technology he presented his vision that physics and engineering could contribute to the development of nanotechnology. Desiring that the information held in various Encyclopedia books could be stored in a small library as a needle tip, Feynman described the beginning of a new technological era that was to materialize by building "molecular" machinery to show the ability to work with a very high atomic precision [67].

What exactly is nanotechnology? This question has failed to define in exact terms the answer, which seems to have multiple variants, therefore, the definition of nanotechnology is one of the confusions generated by this field. The most commonly encountered definitions of nanotechnology revolve around the study of technology that develops nanomaterials and their functionality on a nanomaterial scale, under controlled conditions. [28]

Nanoparticles have captured the interest of researchers over the past few years, due to their special properties, which are clearly superior to the same materials, but in their raw form. These formations, with dimensions around 100 nm, are considered to be the main ingredient in the development of nanotechnologies. It has been shown that the potential of nanoparticles is of a large scale, being used favorably in fields such as medicine, electronics, optics, including the catalysts sector [14].

For a more convenient understanding of the term "nano", comparisons made with human hair, which typically have a diameter of 80,000 nm, are frequently encountered. Another parallel used is a sheet of paper, which is approximately 100,000 nm thick [103]. Also by comparison, a human DNA strand has dimensions of 2.5 nm in length [230]. In nanotechnology, a particle is defined as a small object that behaves as a whole unit in terms of transport and its properties. The term "nanoparticle" does not apply to individual molecules, usually referring to inorganic materials [250].

Metal nanoparticles fascinated scientists for almost half a century, being used in biomedical and engineering sciences. They are a point of interest because of their huge potential in nanotechnology. Today these materials can be synthesized and modified with different functional chemical groups that allow them to be conjugated to antibodies, ligands and drugs of interest, thus opening up a wide range of potential applications in biotechnology. [147]

## 2. The aim of the doctoral thesis

The purpose of this paper is the study of metal nanoparticles, highlighted by their synthesis, characterization and applicability, designed to develop and improve the synergism of antimicrobial activity in combination with certain commercial drugs.

The aim of this thesis is to improve the antimicrobial properties of nanoparticles obtained by the microorganism-mediated green synthesis method.

The specific objectives of the research theme are:

- Critical analysis of the current state of the art of nanoparticle acquisition through top-down approaches and bottom-up approaches.
- Synthesis of metal nanoparticles using reducers and natural stabilizers provided by different microbial cultures.
- Improving the properties and performance of nanoparticles obtained by modifying process parameters (temperature, incubation period, molar concentration, etc.).
- Evaluation of the antimicrobial activity of the obtained nanoparticles
- Develop synergism between nanoparticles and different antibiotics.

The above-mentioned objectives illustrate the content of an interdisciplinary work, which, like nanotechnology, also includes elements in the fields of chemistry, physics and biology.

## 3. The motivation, innovation and importance of this research thesis.

The background on which the thesis is structured is primarily justified by the exponential progress of nanotechnology supported by national and international trends, with a complement to the medical field. Also, the interdisciplinary character of the paper is supported by the benefits of using the green nanoparticle synthesis, such as the low cost of synthesis, the non-polluting elements involved, and the relative ease of industrial scale deployment, not requiring special process parameters (temperatures, pressures, high energy consumption, etc.).

The novelty elements found in this work are mainly represented by the microorganisms that have been used to mediate the nanoparticles synthesis process, as well as certain process parameters that have influenced the final properties of nanomaterial materials.

According to the Web of Science, studying the subject of nanotechnology for the last 5 years, the most common areas of research are Science of Technology and Materials Science that surpasses the field of research in Engineering, Chemistry, Physics and Biology. The type of the most common documents made on the above mentioned topic is concretized in scientific articles, followed by books, patents and news. At a Romania-level, the field of Materials Science aims especially at the development of nanotechnologies.

A particularly important aspect of the topic described is the ability to obtain and influence the properties of nanometric materials due to their size and shape. This has led to an expansion of the nanoparticle application domains in almost all industries and scientific research.

The PhD thesis consists of 6 (six) chapters, 10 appendices. During the course of the thesis, I have approached about 250 bibliographic resources, which mostly contain articles published in specialized journals and books.

*The first chapter* (I), Nanotechnologies and nanomaterials; methods of obtaining, fields of application, aims to highlight and classify some methods of synthesis of metal nanoparticles. These are described briefly to focus on the main elements that define each method, so that there is a simpler selection of them depending on the availability or the results to be achieved. Their classification is primarily based on the principles of top-down and bottom-up approaches. It highlights the method of green synthesis, developed in the present paper, with its advantages and disadvantages.

*The second chapter* (II), Methods of Analysis and Characterization of Structural and Morphological Properties of Metallic Nanoparticles, provides a perspective on the principles of the characterization methods of the nanoparticles used for the PhD thesis.

*In the third chapter* (III), Researches on Biosynthesis and Characterization of ZnO Nanoparticles, ZnO NPs were synthesized using the *Enterobacter* strain LA9 supernatant in combination with zinc nitrate hexahydrate in a concentration of 2 mM , used as a precursor in the synthesis process of zinc oxide nanoparticles. Synthesized solutions containing nanoparticles were characterized by ultraviolet-visible (UV-vis) spectroscopy, resulting in a peak at 368 nm. The energy dispersion X-ray spectroscopy (EDX) confirmed the elemental composition of the zinc oxide nanoparticles. Scanning Electron Microscopy (SEM) showed ZnO NPs with spherical shape, with a diameter of approximately 100 nm and microflora structures after a longer sample incubation period. In this part of the thesis the importance of the process parameters and their influence on the obtained results was demonstrated.

*The fourth chapter* (IV), Researches on Biosynthesis and Characterization of Ag Nanoparticles is dedicated to the biosynthesis of silver nanoparticles using AgNO3 as a precursor by two Bacillus species, namely *Bacillus amyloliquefaciens* and *Bacillus subtilis*. Following the synthesis steps, the absorption of solutions containing brown colloidal nanoparticles was assessed by ultraviolet-visibility (UV-vis) spectrophotometry, which showed the peak absorption values at 418 nm and 414 nm surface plasmon resonance of silver nanoparticles. Analyzes such as EDX, SEM, and Dynamic Light Scattering (DLS) have confirmed the formation of spherical silver nanoparticles with a mean diameter of less than 140 nm. X-ray diffraction (XRD) confirmed the presence of silver crystals with centered cubic structures with the highest peak intensity at 20 = 38.12 ° corresponding to the diffraction patterns (111). Antibacterial activity was observed after 24 hours of incubation against Gram negative bacteria: *Escherichia coli, Pseudomonas aeruginosa, Salmonella* as well as gram positive: *Staphylococcus aureus, Streptococcus pyogenes*. Furthermore, antifungal activity was evaluated against *Candida albicans*. The inhibition zone was clearly seen on silver-containing nanoparticles, either separated or in combination with antibiotics, thus demonstrating their potential antibacterial effect.

*In the fifth chapter* (V), Researches on Biosynthesis and Characterization of AgCl nanoparticles, I presented the results of research on the biosynthesis of silver chloride nanoparticles. They were synthesized using *Rhodotorula Mucilaginosa, Enterobacter Faecalis, Pantoea* and *Raoultella Planticola* microorganisms and AgNO3 aqueous solution as a precursor. The plasmonic resonance of the nanoparticle-containing solution showed a UV absorption maximum at about 440 nm by UV-vis spectrophotometry (UV-vis). Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Dispersive Energy Spectroscopy and X-ray Diffraction (XRD), Atomic Force Microscopy (AFM), and Selected Area Electron Spectroscopy (SAED) the presence of spherical silver chloride nanoparticles with a centered cubic crystal cubic structure and a mean particle size of approximately 10-50 nm. Also, in this study we have demonstrated that silver chloride nanoparticles have the ability to inhibit the growth of various microorganisms such as *Staphylococcus aureus, Streptococcus Pyogenes, Salmonella* or *Bacillus amiloliquefaciens* [89].

At the end of the paper, *in the sixth chapter* (VI), the final conclusions, original contributions, future research directions, and dissemination of the results obtained during PhD studies are presented.

The novelty elements encountered in the synthesis processes presented in the thesis constitute an important basis for the development of future nanotechnology research.

# CHAPTER I
# NANOTECHNOLOGIES AND NANOMATERIALS; SYNTHESIS METHODS, FIELD APPLICATION

Synthesis of metal nanoparticles is an active area of academic research and, more importantly, nanotechnology application. Several methods have been developed for the synthesis of these materials. Techniques for synthesizing nanoparticles can be divided into solid, liquid and gaseous phase processes [81].

Due to the size of nano-scale materials, their behavior is remarkable. The properties of the materials are primarily influenced by the increase in surface / volume ratio, which results in an increase in the total surface and the fraction of entities as shown in Figure 1.1. Electrical, magnetic, solubility or reactivity properties are superior to those of the same larger particle size materials [11].

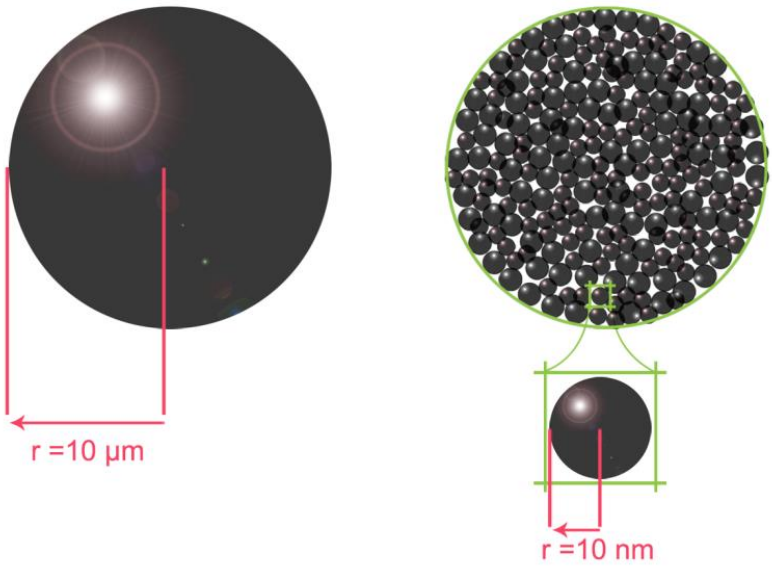



Figure 1.1 Schematic representation of surface / volume ratio change between a microsphere (stg) and the same volume of microsphere, but composed of nanoparticles (dr) [26]

In recent years, nanoparticle synthesis has gained interest due to potential application in several areas, such as medicine, the catalyst industry, and biosensors [189], [211]. Among the most important and best studied types of metal nanoparticles were noble metals (gold and silver), cadmium sulfide, copper, zinc oxide and titanium dioxide [104],[116],[182] [193],[215].

Nanocrystalline materials can be synthesized either by reinforcing atoms / molecules / atomic groups or by decomposing large-scale material to smaller sizes. In Figure 1.2 some of the synthesis methods of nanoscale structures that include both top-down and bottom-up approaches [5], [ 146].

Synthesis methods of nanoparticles may generally involve either a top-down approach or a bottom-up approach, in their turn, the methods being classified as in Figure 1.3.

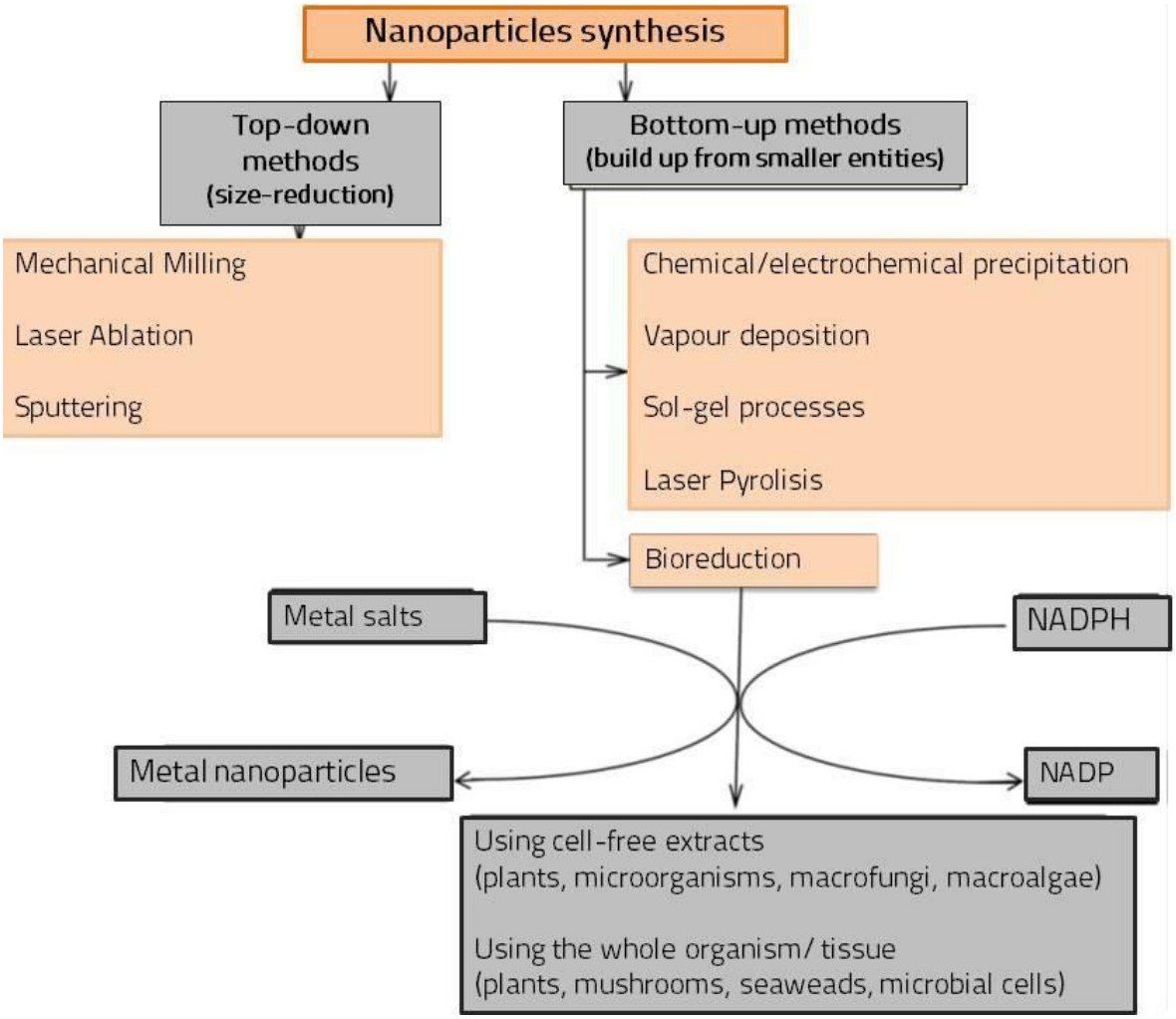



Fig. 1.3 Different Approaches to Manufacturing Nanoparticles [5], [146].

## 1.1 "Top-down" methods

In this category, top-down synthesis, nanoparticles are obtained by reducing the size of macroscopic systems to nanoscale. The reduction in particle size can be achieved by different physical or chemical procedures when applying a source of energy, which may be mechanical, chemical or thermal. It is also possible to use another form of energy, such as laser irradiation [5], [92].

## 1.2 "Bottom-up" methods

In "bottom-up" synthesis processes, the individual manipulation of atoms and molecules through self-assembly processes leads to the formation of nanostructures. This approach can also be materialized by applying biological methods. The precursor used is usually a liquid or gas that is ionized, dissociated, sublimed or evaporated and then condensed to form amorphous or crystalline nanoparticles [61], [92], [48].

The advantages of these techniques consist of a homogeneous chemical composition, a low particle size variation, and the number of nanoparticle surface defects, considerably lower than with top-down approaches [92].

## 1.2 Green synthesis of metallic nanoparticles

In recent years, biological synthesis has emerged as an appealing alternative to traditional nanoparticle production methods. Biosynthesis involves green approaches based on green methods using single and multicellular biological entities such as bacteria, actinomycetes, fungi, plants and yeasts or plant extracts [144].

In addition to the use of microbes and plants, green methods of synthesis currently include different approaches through the use of biological materials like honey, starch or ascorbic acid. They have been used so far to synthesize gold, silver, palladium, carbon and platinum nanoparticles [19].

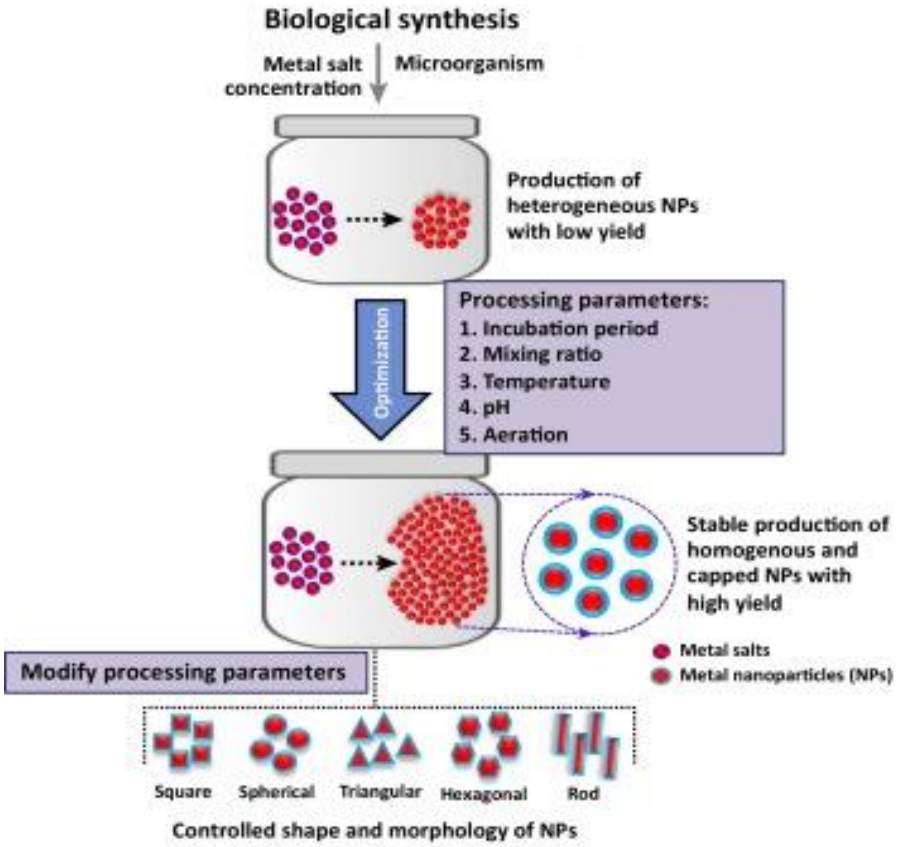



Fig. 1.16 Biological synthesis of metallic nanoparticles using microorganisms [195]

Although microorganisms and plant extracts can be used to synthesize metal nanoparticles, it is very important that the process will be optimized to produce homogeneous nanoparticles of similar size and shape. This can be done by adjusting the control parameters, such as the precursor

concentration, the mixing ratio between the biological extract and the metal salt, pH value, temperature, incubation time and aeration [210], [78].

### 1.3.1. Green synthesis using plant extract

Plants were considered a more ecological way for the biological synthesis of metallic nanoparticles. They have potential in the hyper-accumulation and biological reduction of metal ions.

Plant extracts contain bioactive alkaloids, phenolic acids, polyphenols, proteins, sugars and terpenoids, which play an important role in the initial reduction of metal ions and then in their stabilization. Variation in the composition and concentration of these active biomolecules between different plants and their subsequent interaction with metal ions contributes to the diversity of nanoparticle sizes and forms [144], [210].

Natural products or those derived from natural products, such as extracts from several plants or parts of plants, tea, coffee, bananas, plain amino acids, as well as wine, table sugar and glucose, have been used as reducing agents and as agents coating during the present synthetic method [210].
Also, recent experiments have revealed the reducing potential of leaf extracts, seed extracts, root extracts, bulbs and plant latex, which are used to synthesize gold, silver and palladium nanoparticles [19].

### 1.3.2 Green synthesis of nanoparticles using microorganisms

Microbes produce inorganic, either intra- or extra-cellular materials, often in nanometer sizes, with a refined morphology. Detoxification may be achieved either by microbial extracellular biomineralization, biosorbtion, complexation or precipitation, either intracellularly by bioaccumulation [38].

Bioreduction consists in the chemical reduction of metal ions in more biologically stable forms. Many organisms have the ability to use disimilator metal reduction, the reduction in metal ion oxidation is coupled to an enzyme. This results in stable and inert nanoparticles that can then be safely removed from a contaminated sample [152].

The most versatile location of nanoparticle biosynthesis is that of biological cells and their cell membrane. Biosynthesis is the phenomenon that occurs through biological or enzymatic reaction [46].

There are two types of biosynthesis, depending on where the process takes place, ie intra- or extracellular. Intracellular synthesis occurs in the cell, while extracellular synthesis occurs due to cell-secreted enzymes [73]. The extracellular production of metallic nanoparticles has many commercial applications in various fields because polydispersity is the major concern. It is important to optimize the conditions of biological processes for obtaining monodispersed nanomaterials [46].

### 1.3.3. Applications of nanoparticles obtained by biosynthesis

Nanotechnology is a growing field of research with a strong impact in all spheres of human life, which is why biological nanoparticle synthesis becomes a safer and better alternative to conventional methods [240].

Nanomaterials are being used successfully in the field of nanomedicine, which has continued perspectives for improving the diagnosis and treatment of human diseases. The dispersed nanoparticles are typically used in nanobiomedicine as fluorescent biological labels, drugs and gene delivery agents and in applications such as biodetection of pathogens, tissue engineering, tumor destruction by heating, and improved NMR contrast. Their use has been explored in applications such as drug delivery, cancer treatment, gene therapy and DNA analysis [220], [179].

Nanoparticles have been widely used to improve various reactions as reducers and / or catalysts in the chemistry field due to their large specific surface area. Nanoparticles synthesized by biological processes have a higher catalytic reactivity and a higher specific surface area [46], [239].

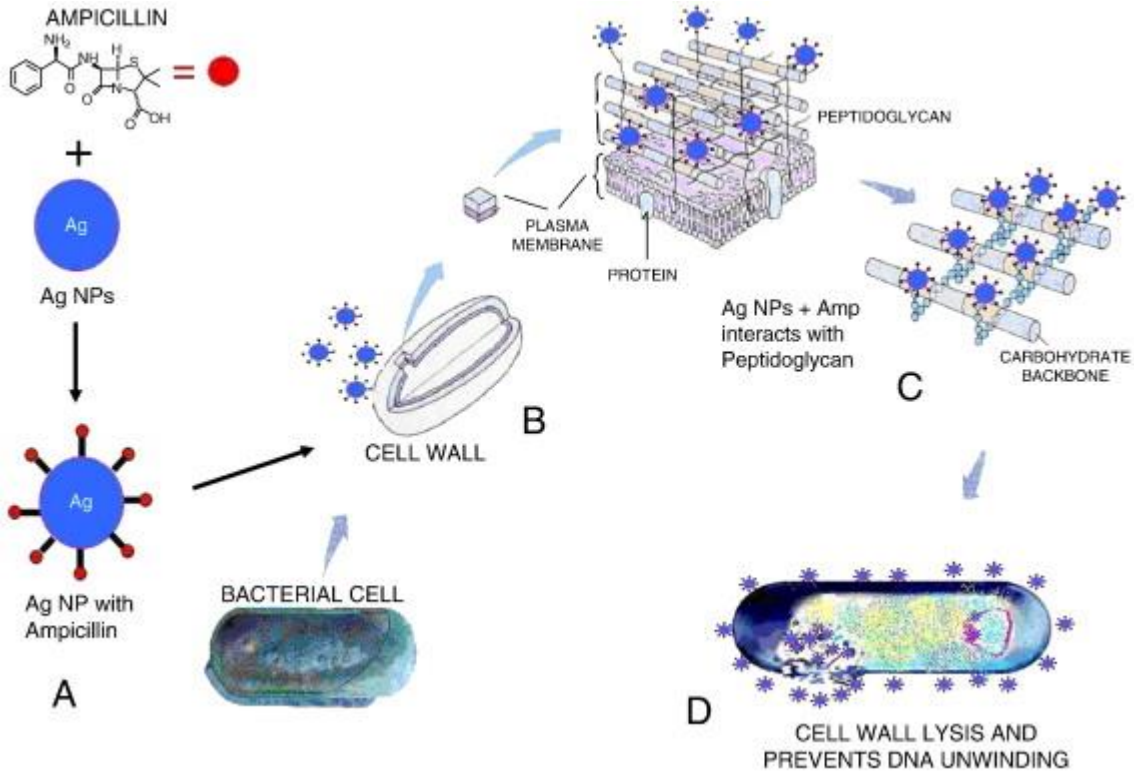



Fig. 1.17. Synergistic activity of AgNP with ampicillin (Amp) against bacteria [65]

Many of the antibiotic-resistant organisms have a sensitivity to antibiotics combined with metal nanoparticles. Fayaz presented a possible explanation for the improvement of the antibacterial synergistic mechanism (Figure 1.17) [51], [65] where the following phenomena appeared:

- The formation of basic silver nanoparticles with ampicillin..
- Interaction between AgNPs-Amp over the bacterial cell wall.
- The AgNPs-Amp complex inhibits the formation of crosslinks in the peptidoglycan layer (which ensures cell wall stiffness), leading to lysis of the cell wall.

- The AgNPs-Amp complex prevents DNA movement

## 1.4 Conclusions

- "Top-down" synthesis methods are being sought to be replaced or improved due to imperfections in surface structure. This is a major limitation because surface chemistry and other physical properties of nanoparticles are strongly dependent on surface structure. In addition, they can contain significant amounts of impurities. They have a relatively wide distribution of the dimensions and a different form of the particles [161], [86].
- "Bottom-up" approaches have proven to be more favorable, which is why a multitude of nanoparticle synthesis techniques have been developed following the principle of self-assembly.
- Nanomaterial synthesis techniques, both top-down and bottom-up have revolutionized the use of nanomaterials in different areas. The potential of nanoparticles to present ecologically stable sizes and shapes increases the demand for industrial scale production.
- Biosynthesis of metal nanoparticles is an interdisciplinary field ("bio-nanotechnology") that requires collaboration between physicists, chemists, biologists and engineers. The exploitation of natural resources and the implementation of these biological synthesis methods have proved to have many advantages, such as: environmental protection, simplicity of implementation in production, cost-effectiveness.
- Lack of a subsequent complex chemical synthesis, lack of toxic contaminants and rapid synthesis capacity using numerous medicinal plants and micro-organisms.
- Biosynthetic production rate and particle monodispersity are continually improved. The transition from bacteria to fungi as a means of developing natural "nanofabrics" is an added advantage in the synthesis of nanoparticles using microorganisms..
- Biochemical pathways involved in the synthesis of nanoparticles need to be carefully studied, and the specific genes and enzymes involved must be characterized. This will help us have better control over the parameters that define the properties of a nanoparticle, such as size, shape, and monodispersity..
- Future research on the microbial synthesis of nanoparticles with unique properties are of great importance for applications in the field of medicine, agriculture and electronics.

# CHAPTER II
# METHODS OF ANALYSIS AND CHARACTERIZATION OF THE STRUCTURAL AND MORPHOLOGICAL PROPERTIES OF METAL NANOPARTICLES.

Metal nanoparticles have found applications for centuries in paints and pigments, but the promise of developing the next generation of electronic and medical devices, chemical sensors and drugs has now been demonstrated. In the last decades, much progress has been made both in the synthesis and characterization of these systems [100].

The synthesis of metal nanoparticles is followed by their characterization properties, such as size, shape, surface morphology and dispersion. In the case of both chemical and green approaches, ion metal salts are reduced, resulting in a change in color in the reaction mixture, which is the first indication that nanoparticles are formed. However, a detailed characterization is required to highlight their formation.

Among the most commonly used techniques for characterization of biosynthetic metal nanoparticles are the following: ultraviolet-visible and visible (UV-vis) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), ray spectroscopy (EDEM), Transmittion Electron Microscopy (TEM), Selected Area Electron Spectroscopy (SAED), Atomic Force Microscopy (AFM), Dynamic Light Displacement Inductively coupled plasma spectrometry ICP-MS) [199], [75].

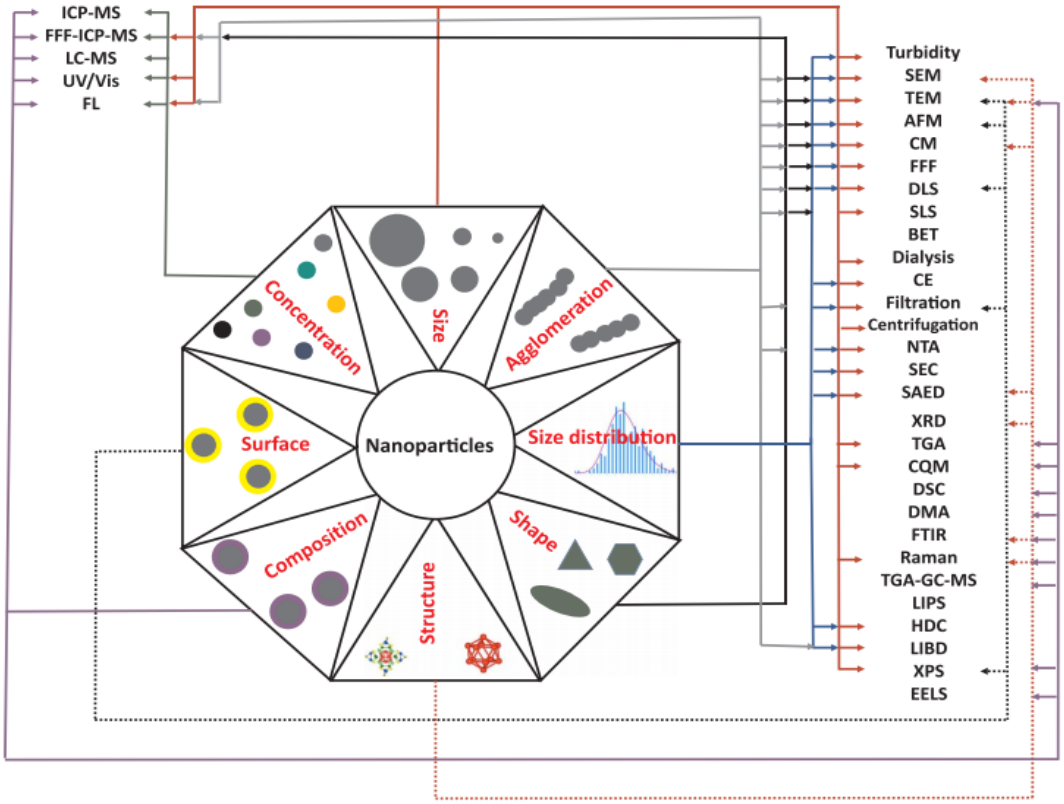



Figure 2.1 Methods of material characterization [199].

In Figure 2.1 various methods of characterizing the chemical and physical parameters (concentration, size, agglomeration, size distribution, shape, structure, composition, and surface) used to explore the "nano" world and the analytical methods used for sizing and quantifying in each case are presented [199].

## 2.8 Conclusions

- The rapid development and production of nanomaterials for their usage in nanomedicine indicates the demand and wisdom to regulate the manufacture and use of nanomaterials. Robust techniques for the characterization of nanomaterials are fundamental to regulatory guidelines for ensuring the safety of nanomaterials in general and nanomedicine in particular..
- The brief description of each technique, along with its strengths and limitations, gives us an insight into the selection of suitable techniques for characterizing particles with potential applications in nanomedicine.
- UV-vis spectroscopy is a simple and cost-effective method for determining the concentration of different samples if applied to pure compounds and used with the appropriate standard curve. A standard curve referring to absorption can be developed for any compound and is used to determine the concentration of samples containing the same compound.
- SEM and TEM offer unique advantages for the very detailed characterization of nanoparticles. The main difference between the results of the two techniques is how the nanoparticle images are obtained. It is important to understand the benefits of each technique before deciding which technique to use.
- DLS is a widely used method for measuring particle size for a truly diverse range of sample types. Along with particle size, light scattering systems can also determine molecular weight, protein loading, and zeta potential. There is a variation in the considerable sensitivity from the system to the system, defining the size of the particles or molecules that can be measured correctly, as well as the measuring capacity at high concentrations.
- X-ray diffraction is a technique for analyzing the crystalline structure of materials. The X-ray beam strikes a crystal, spreading a beam in a manner characterized by the crystalline structure. Even complex structures by X-ray diffraction, such as DNA and proteins, can be analyzed.

# CHAPTER III

# RESEARCHES ON BIOSYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES

## 3.1 Contributions to the study of ZnO particles

Zinc oxide nanoparticles (ZnO NPs) have diverse applications in the fields of electronics, chemical sensor, personal-care products and food industry. In this study, zinc oxide nanoparticles (ZnO NPs) were synthesized using cell extract of Enterobacter. sp. LA9 with 2mM concentration of zinc nitrate hexahydrate which was used as zinc oxide precursor.

The synthesized nanoparticles were characterized by ultraviolet-visible spectroscopy which showed a peak at 368 nm. Energy Dispersive X-ray Spectroscopy analysis confirmed the elemental composition of zinc oxide nanoparticles. Scanning Electron Microscopy showed spherical shape ZnO NPs, with diameter of less than 100 nm [79].

The aim of this chapter is to examine the synthesis of ZnO NPs by Enterobacter sp. LA9 and to point out the conditions that ZnO NPs can be formed as well as the characacteristsics of ZnO NPs. Interestingly, this would be the first instance of ZnO NPs synthesis using Enterobacter species.

## 3.2 Synthesis of nanoparticles and micro-flower structures of zinc oxide

### 3.2.1 Bacteria and culture conditions

The Enterobacter sp. LA9 was previously isolated from oil-contaminated site and characterised by 16S rRNA at the at the Dep. of Environmental Science and Technology at Cyprus University of Technology. The *Enterobacter* sp. LA9 showed high production of Extracellular Polysaccharides (higher than 1500 mg Dry weight EPS/L) and emulsification activity (higher than 70% in olive oil). The pure colonies of isolate were inoculated on petri dishes containing 1g/L yeast extract; 18 g/L agar-agar; 5 g/L sodium nitrate; 0.2 g/L glucose. The inoculated plates were incubated at 33°C for 48 h. For precursor, zinc nitrate hexahydrate from Sigma-Aldrich was used.

### 3.2.2 Biosynthesis of ZnO NPs using *Enterobacter* sp. LA9

The Enterobacter sp. SW culture was grown in liquid media containing 5 g/L NaCl, 6 g/L glucose, 1.8 g/L yeast extract, 1 g/L H2KO4P, 0.6 g/L peptone, 1g/L NaNO3, culture which was used for nanoparticles synthesis. After 2 days of keeping the culture in the incubator at 33°C, at 100 rpm, the supernatant (liquid culture) was separated from the solid biomass by centrifugation at 4000 rpm, 10°C for 30 min and passed through 0.45 μm filter.

The solution containing 2mM of precursor $Zn(NO_3)_2 \cdot 6H_2O$, was prepared using distilled sterile water, heated in oven at 80 ◦C for 30 min and put on orbital shaker for 2h at 100 rpm. The samples were prepared in ratio 1:9 (supernatant: precursor).

The samples were kept in conical flasks in the incubator at 33°C, 100 rpm. After 72 hours, the solution was dried in the oven at 80◦C. Following this step, the solution was washed five times with distilled sterile water in order to remove impurities. In the end, crude pellets were then re-suspended in 5 ml sterile distilled water. 4ml of solution was added in a ceramic cup and kept in the oven overnight.

First sign of formation of zinc oxide nanoparticles can be observed in Fig.1-a, where the white deposition from the bottom of the flask, indicate the presence of this kind of nanoparticles [17]. It is significant to take into account that this sign can be deceptive because the particles can be bigger in size, therefore not necessarily considered nanometric, while showing the same white deposition.

## 3.3 Characterization of structures obtained and interpretation of data. Used equipment.

### 3.3.1 Plasmonic resonance of ZnO nanoparticles (UV-visible)

The possible bioreduction of ZnO NPs solution was monitored by periodic sampling. UV-vis spectra of 1ml aliquots were monitored as a function of time of reaction on a Lambda 25 spectrophotometer in the 300-700nm range operated at a resolution of 1nm. For this analysis distilled water as the control was used. The data of interest was the maximum peak position and intensity.

Figure 1b represents the variation in absorbance of the prepared solution, as function of the radiation wavelength. The time incubation, from 48 to 72 hours, presents an absorbance increasing. A peak at a wavelength 368 nm can be observed, indicating the formation of zinc oxide nanoparticles [79].

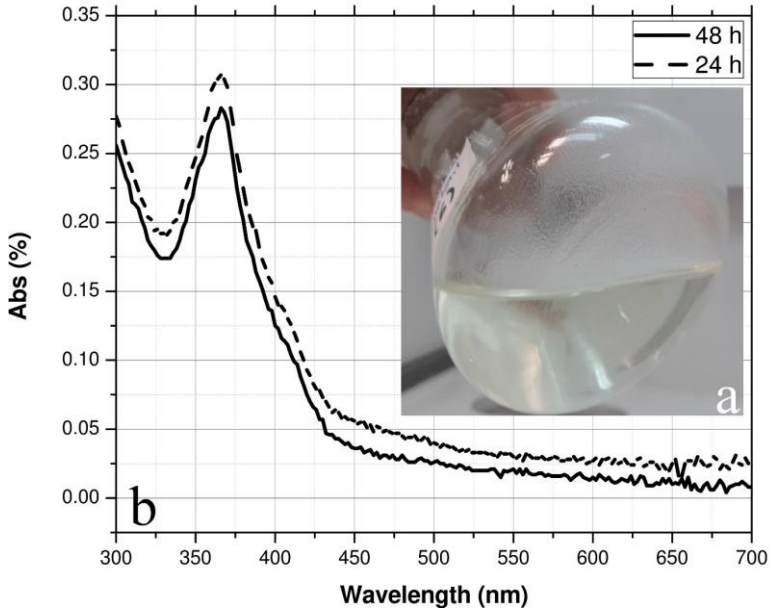



Figure 3.1 UV-vis spectroscopy of synthesized ZnO NPs with peak at 368 nm

### 3.3.2. Structural and Morphological Analysis of Particles by Scanning Electron Microscopy (SEM)

In order to assess the particle size and morphology, surface evaluation was performed using a Quanta 200 Scanning Electron Microscope (SEM; FEI, Hillsboro, OR) equipped with Energy Dispersive X-ray Spectroscopy (EDS) that allowed for chemical composition analysis.

The challenges during biosynthesis include optimal production and minimal time to obtain the desired size and shape, to enhance the stability of nanoparticles and optimization of specific microorganisms for specific application. Figure 3a shows the zinc oxide nanoparticles of spherical shape (the most common shape of metallic nanoparticles) synthesized by Enterobacter sp. LA9. It can be noticed that the size is predominantly smaller than 100 nm.

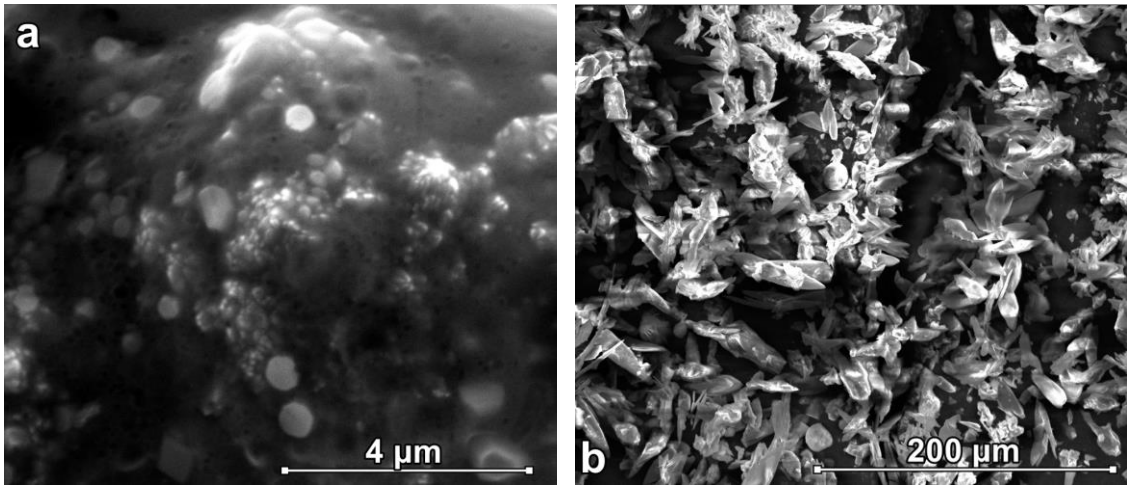



Figure 3.2 - a SEM micrographs of nanoparticles ZnO: spherical (a);
micro-flowers structures (b).

### 3.3.3. Determination of the chemical composition by X-ray dispersive energy microanalysis (EDX)

EDS analysis verified the chemical composition for the biosynthesized zinc oxide nanoparticles (Figure 2a) and zinc oxide micro-flowers (Figure 2b) using Enterobacter sp. LA9. The both recorded spectrums present strong signals of Zn and O.

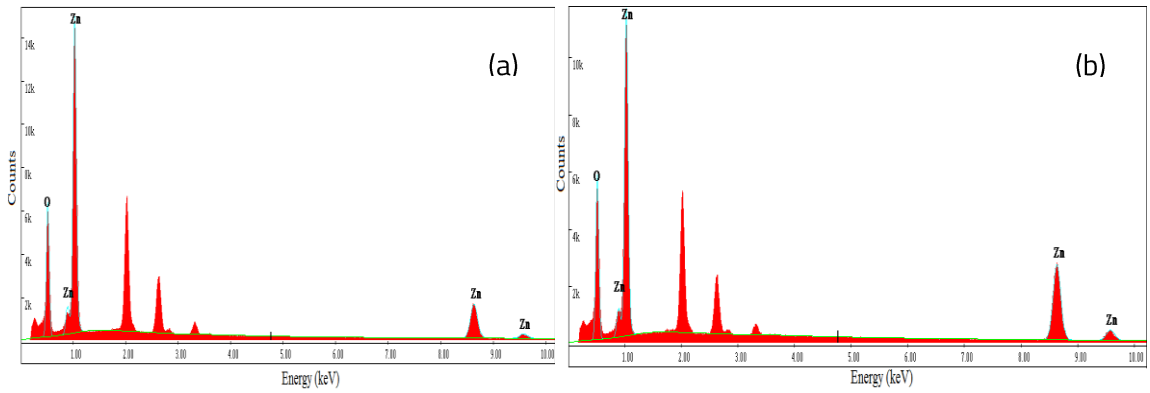



Figure 3.3 EDS spectra of zinc oxide nanoparticles (a); zinc oxide micro-flowers (b).

In order to obtain different properties and characteristics of zinc oxide nanoparticles we changed one parameter at the time of the process. Significantly different results were noticed when the samples were incubated for 48 hours instead of 72 hours. The white deposition was present, the peak was around the same wavelength, but the size and shape were totally different.

Meghana Ramani et al.[167] have demonstrated the synthesis of bundles of ZnO microflowers from nanoparticles by a simple wet chemical route. In that case the shape of ZnO evolved from nanoparticles into bundles of micro-flowers.

Zinc oxide microflowers were obtained by changing the parameter: the incubation time of the samples including zinc nitrate (Fig. 3b). This morphology of ZnO materials could be of major interest, mainly due to its good photocatalytic activity, considering the significantly larger surface area, compared to the spherical particles [222].

## 1.4. Conclusions

- The synthesis of metallic nanoparticles using microorganisms is a cost-effective and eco-friendly method. The attractive features of this protocol are simple reaction procedure, short reaction time and easy products.
- The zinc oxide nanoparticles obtained by extracellular synthesis using *Enterobacter* sp. LA9 and Zn(NO3)2·6H2O like precursor, has been shown to be feasible. The step from zinc oxide nanoparticles to micro-flowers, changing only one parameter (incubation time), can broaden the range of possible applications.
- The beginning of the formation of zinc oxide nanoparticles is a little harder to identify because of the white deposition easily visible to the naked eye.
- The method of synthesis used is environmentally friendly.
- Zinc oxide nanoparticles have important applications in cosmetics, of which sunscreen creams are of interest to researchers.

# CHAPTER IV
# RESEARCHES ON BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES

## 4.1 Contribution to the study of Ag nanoparticles

This chapter is dedicated to biosynthesis presentation of silver nanoparticles, using $AgNO_3$ as a precursor, by two Bacillus species, namely *Bacillus amyloliquefaciens* and *Bacillus subtillis.*

Following the synthesis steps, the absorption of brown colloidal solutions containing nanoparticles was evaluated by ultraviolet-visibility (UV-vis) spectrophotometry, which showed the peak absorption values at 418 nm and 414 nm surface plasmon resonance of silver nanoparticles [75].

Analyzes such as Energy Dispersive Spectroscopy (EDX), Scanning Electron Microscopy (SEM), and Dynamic Light Scattering (DLS) have confirmed the formation of spherical silver nanoparticles with a mean diameter of less than 140 nm. X-ray diffraction (XRD) confirmed the presence of silver crystals with centered cubic structures with the highest peak intensity at 20 = 38.12 ° corresponding to the diffraction patterns (111).

Antibacterial activity was observed after 24 hours of incubation against Gram negative bacteria: *Escherichia coli, Pseudomonas aeruginosa*, *Salmonella* as well as gram positive: Staphylococcus aureus, Streptococcus pyogenes. Furthermore, antifungal activity was evaluated against *Candida albicans*. The inhibition zone was clearly observed on disk containing silver nanoparticles, either separated or in combination with antibiotics, thus demonstrating their potential antibacterial effect.

## 4.2 Silver nanoparticles synthesis

### 4.2.1 Bacteria and culture conditions

*Bacillus amyloliquefaciens* 1853 and *Bacillus subtillis* 10833 used in this study were provided by the University of Technology in Cyprus. The bacteria were grown in the following solid medium: 1 g / l yeast extract; 18 g / l agar-agar; 5 g / l sodium nitrate; 0.2 g / l glucose (all supplied by Scharlau Chemicals), which was sterilized at 128 ° C in the volume of 100 ml of distilled water. The cultures were inoculated into solid media into 55 mm diameter petri dishes in 12 ml of medium and after gelling were inoculated with 1 μl of each bacterium and subsequently incubated at 33 ° C for 48 hours.

### 4.2.2 Biosynthesis of Ag NPs using *B. amyloliquefaciens* 1853 and *B. subtillis* 10833

To synthesize silver nanoparticles, 1 μl of bacterial strains were freshly inoculated into conical flasks containing 100 ml of liquid medium (0.6 g / l yeast extract, 1 g / l sodium nitrate, 3 g / l glucose) to 33 ° C for 48 hours.

After 48 hours, the cultures were centrifuged at 4000 rpm for 30 min using an angular rotor centrifuge. 10 ml of the supernatant was mixed with 90 ml of precursor (1 mM aqueous solution of AgNO3). The precursor was first autoclaved (sterilized) at 128 ° C for 30 minutes. The procedure is schematically shown in figure 4.1.

The supernatant and the precursor, in the present case, silver nitrate, were kept for control. Samples were incubated for 48 hours at 33 ° C (to ensure maximum enzymatic activity of the extract) and 150 rpm. In addition, a control sample containing the precursor and the fresh, bacterial-free liquid medium was prepared to evaluate its behavior with respect to nanoparticle synthesis. The control sample was kept under the same conditions as samples containing the supernatant.

For purification of biosynthesized Ag NPs, the samples were centrifuged at 4000 rpm for 30 min. The collected pellets were washed with 25 ml of distilled water and dried at 80 ° C until the liquid was evaporated. This last step was performed 4 times, then the samples were dried in an oven at 200 ° C for 6 hours.

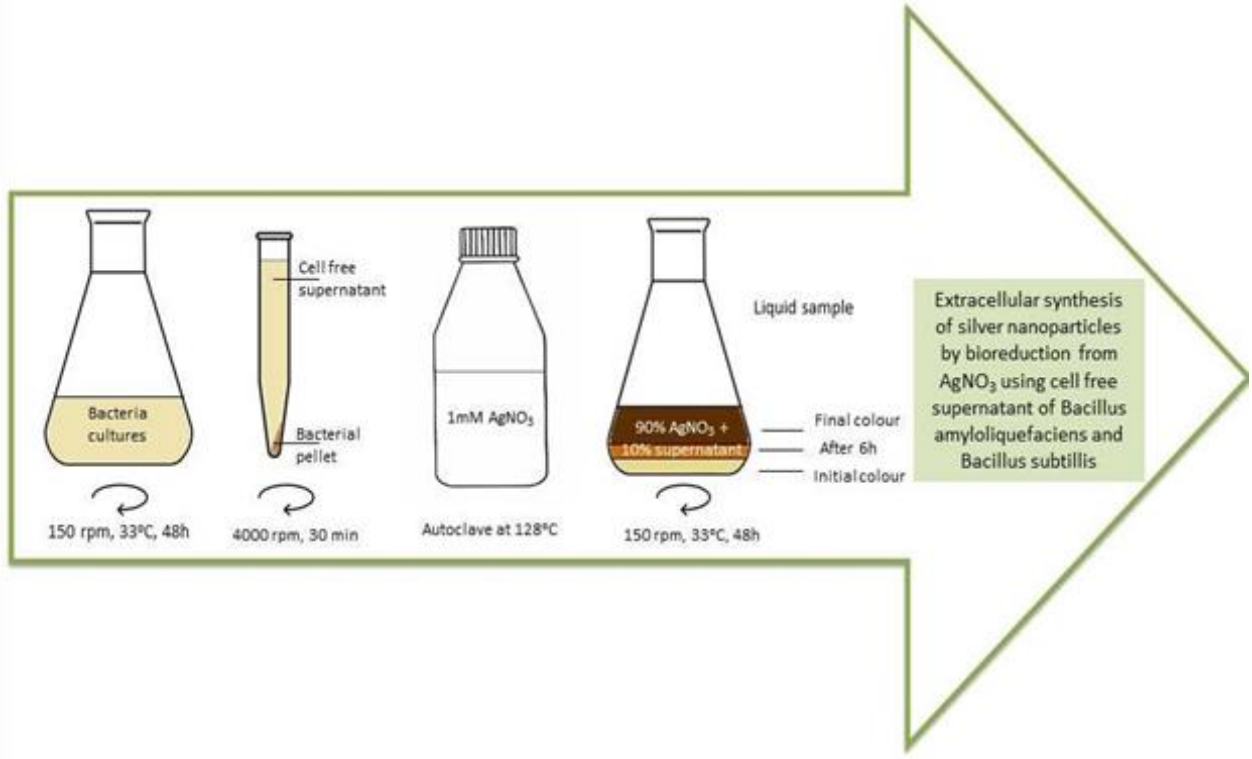



Figure 4.1 Synthesis procedure of silver nanoparticles, using the supernatant as reducer and silver nitrate as precursor [75].

The mechanism of synthesis of Ag metal nanoparticles could be as follows:

$Ag^+$ ions are reduced to $Ag^0$ atoms in the presence of aqueous enzymatic extracts from the Bacillus species supernatant as described in Equation 4.1:

$$Ag^+ + e^- \text{ (enzime)} \rightarrow Ag^0 \qquad \text{(ec. 4.1)}$$

The starting point for the formation of silver nanoparticles was observed after 6 hours, when the color of bacterial precursor-bacterial extract samples began to change from pale yellow to light brown due to the surface plasmon resonance phenomenon [2]. After 48 hours of incubation, the final color of the samples was changed to brown (Figure 4.2). This was the first sign of the extracellular synthesis of silver nanoparticles, taking into account that only the culture supernatant was used.

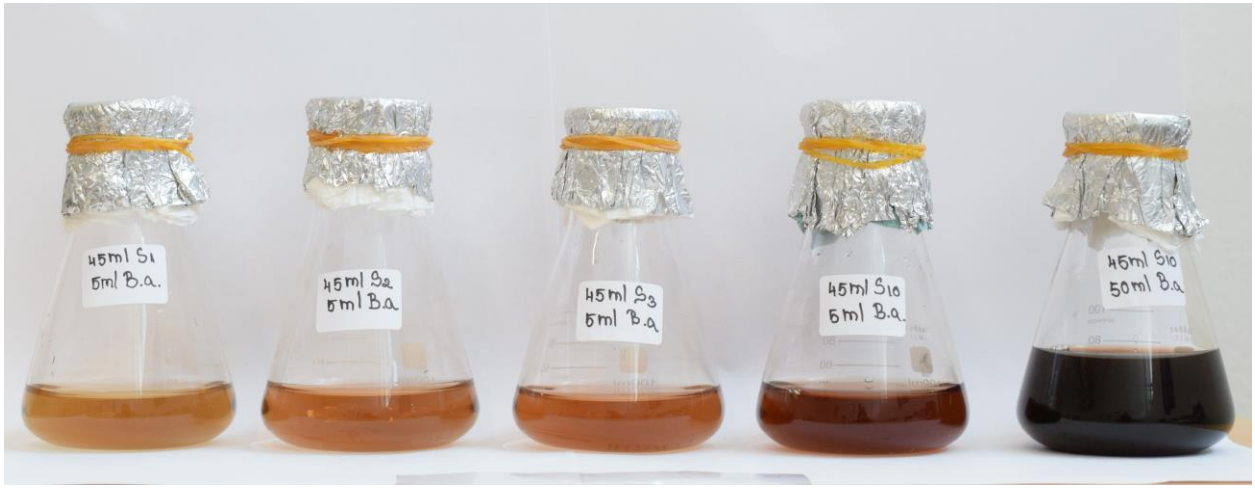



Fig. 4.2 Color variation of the colloidal solution, depending on the incubation period.

Reduction of silver nitrate could be produced by cellular supernatant constituents. Peptides or proteins may be responsible for reducing Ag + ions and subsequent formation of silver nanoparticles. It has been shown that the synthesis of silver nanoparticles can be mediated by the alpha-amylase enzyme, both of which are well-known Bacillus species as manufacturers of such enzymes [37], [75].

Up to now, it has been reported that *B. amyloliquefaciens* (LSSE 62) is capable of synthesizing Ag NPs in the presence of solar irradiation [227]. Moreover, the biosynthesis of AgNPs using the B. subtillis supernatant (ATCC 6633) was reported [178], the estimated duration of the 5 day process being significantly longer than in the results presented here.

An indication of the complete reaction (i.e., biotransformation of the entire amount of $Ag^{+}$ from the precursor solution into Ag NPs) was evaluated by the Mohr titration method [120] using 10 ml of standard 1 mM KCl, 0.5 ml of 0.25 M indicator. K2CrO4 and the AgNO3 precursor solution, respectively the colloidal AgNP solution as the titrant. While in the $AgNO_3$ precursor solution (1 mM, with a factor of 0.980), the $Ag_2CrO_4$ red-brine precipitation color was observed in the solution (Figure 4.3), for colloidal AgNP solutions synthesized with the two bacterial supernatants, no precipitation occurred, indicating a complete reduction of Ag + to Ag0. The Mohr's sensitivity is [Ag +] = 1,35 × 10-5 M [42], so it can be assumed that the reduction reaction is virtually complete (the bacterial enzyme extract is excess) [67].

Considering the complete bio-reduction of Ag +, it could be considered that the same concentration of Ag NPs exists in both colloidal solutions of *Bacillus amyloliquefacien*s and *Bacillus subtilis* colloidal solutions, namely 8.82 × 10-4 moles Ag NPs / l or 0.09513 mg of Ag NPs / ml.

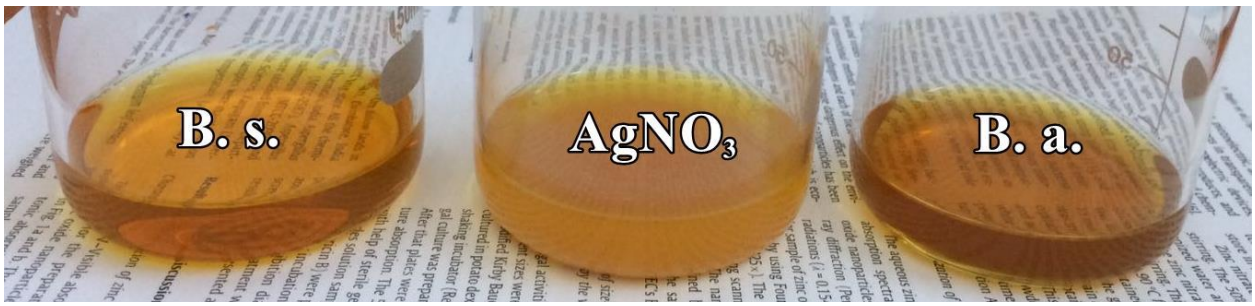



Fig. 4.3 Photographic images of precursor solution after titration and colloidal AgNP solutions, respectively. Lack of precipitation is an indicator for the complete reduction of $Ag^{+}$ la $Ag^{0}$

## 4.3 Characterization of structures obtained and interpretation of data. Used equipment

### 4.3.1 Plasmonic surface resonance of Ag nanoparticles

The UV-VIS absorbance spectra were obtained by using an Infinite 200 microplate plate reader from Tecan using 250 μl of aqueous solution, placed on Greiner 96 flat bottom transparent polystyrene plates. The UV-visible spectra were measured in the range 300-600 nm with a wavelength step size of 2 nm at a temperature of 25 °C.

Figure 4.4 shows the UV-VIS spectra of the solutions containing AgNPs obtained with the supernatant of Bacillus species. The surface plasmon resonance peaks were observed at 418 nm and 414 nm for silver reduced by *Bacillus amyloliquefaciens* and *Bacillus subtillis*, respectively. Those peaks correspond to the plasmon resonance of silver nanoparticles, according to other reports, which present a peak around 420nm [75]

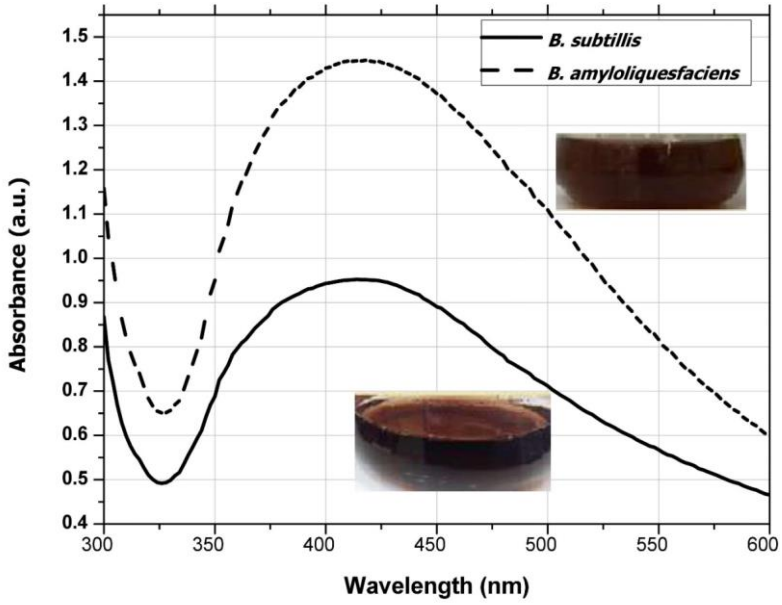



Figure 4.4 UV-vis absorption spectra of colloidal AgNP solutions having similar plasmon resonance peaks.

Some differences regarding the maximum intensity absorbance peak position for solutions containing silver nanoparticles, obtained using the same species of Bacillus, but different process parameters, including solar irradiation and microwave are reported elsewhere: by using *B. amyloliquefaciens* the absorbance has decreased from 423 nm to 418 nm [178], while the absorbance of AgNPs colloids using *B. subtillis* has been presented to have higher values from 410 nm to 414 nm [214],[75]. Those aspects show the first sign of different results regarding the geometry (shape, size) of the nanoparticles. Generally, a longer wavelength for the absorbance peak position would indicate the formation of bigger particles [75],[164]

From figure 4.4 it could be seen that at roughly the same Ag NPs concentration, different values for the absorbance at maximum absorption wavelength could be registered, the NPs colloidal solution synthetized with *B. amyloliquefaciens* having a 35% higher absorbance value than in the case of *B. subtillis*. This could be explained by the multiple scattering theory, which relates the experimental absorbance values (A) to particle radius (R), density of particles per unit volume (N), according to eq. 2. [95]:

$$A = \frac{\pi R^2 Q_{ext} d_0 N}{2.303} \qquad \text{(ec. 4.2)}$$

In eq. 2 $Q_{ext}$ is the extinction coefficient (assumed constant for a type of NP) and $d_0$ is the optical path length of the spectrophotometer (kept constant for all measurements).

The geometric mean radius of the NPs ($R_{mean}$) has been determined from DLS (fig.7a and b) with eq. 3:

$$R_{mean} = \frac{\sum_{i=1}^{n} (I_{DLS,i} \cdot R_{DLS,i})}{\sum_{i=1}^{n} I_{DLS,i}} \qquad \text{(ec. 4.3)}$$

where $I_{DLS,i}$ represents the relative dynamic light scattering (DLS) intensities and $R_{DLS,i}$ the corresponding NP radius ascribed to each DLS maximum.

The density of nanoparticles per unit volume (N) has been calculated by the method proposed by Turkevich [45] as follows:

$$N = \frac{m_{Ag,synt}}{\rho_{Ag} \cdot V_{NP}} \qquad \text{(ec. 4.4)}$$

where $m_{Ag,synt}$ represents the mass of silver ions from the precursor solution (9.513 mg), $\rho_{Ag}$ represents the density of silver (10.49 g/cm$^3$) and $V_{NP}$, the volume of a single nanoparticle (assumed spherical).

$$V_{NP} = \frac{4\pi R_{mean}^3}{3} \qquad \text{(ec. 4.5)}$$

The $R_{mean}$ = 31.11 nm and N = 7.19 × 10$^{11}$ for the NPs synthesized with *B. amiloliquesfaciens*, while for B. subtilis $R_{mean}$ = 58.54 nm and N = 1.07 × 10$^{11}$. As the $R_{mean}^2 \times N$ product value is 72% higher for *B.*

*amiloliquesfaciens*, higher values for absorbance are expected for this colloidal AgNPs solution, in accordance with the experimental UV-VIS spectra.

### 4.3.2 Structural and Morphological Analysis of Particles by Scanning Electron Microscopy (SEM). Determination of chemical composition by X-ray microanalysis (EDX)

A JSM 7400f scanning electron microscope (SEM) was used for surface morphology and chemical composition analyses by Energy Dispersive Spectroscopy (EDS). The silver nanoparticles powder was mounted on the sample holder using double-sided adhesive carbon tape. The acceleration voltage was fixed to 10kV.

The quantitative elemental analysis shows that the silver content is predominant. The other elements shown on the EDS spectrum (fig. 4.6.) appear mostly due to the impurification of the samples from two sources: firstly, due to the carbon tape used for mounting the nanoparticles on the sample holder, secondly due to possible remnants from the media used for the growth of the bacteria. This last situation can be avoided either by reducing the amount of glucose and sodium nitrate in the liquid media, or by performing a calcination process, prior to sample mounting. The Ag NPs were found to have spherical shape with dimension values in good agreement with the results from DLS, according to the SEM micrographs (fig.4.8, fig.4.9).

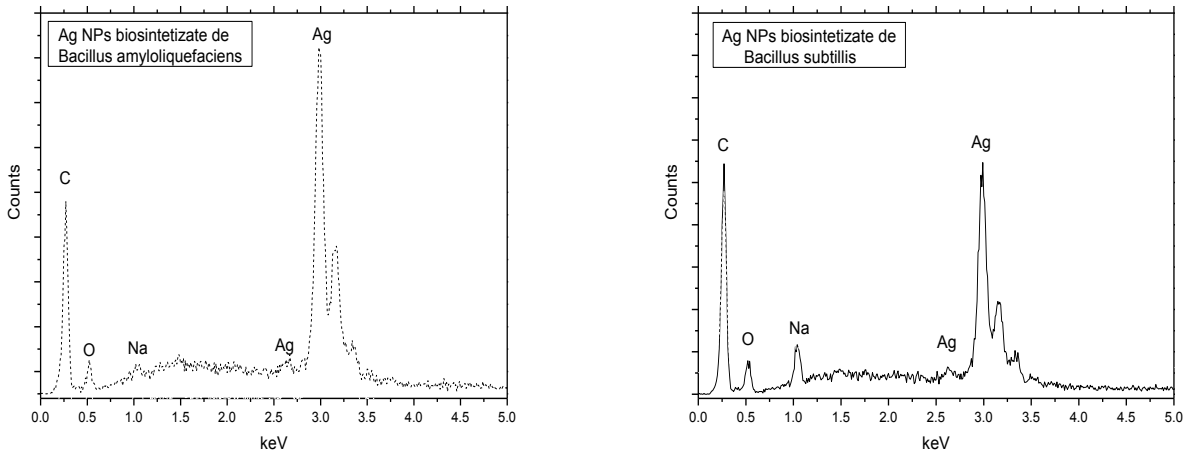



Fig.4.6 Energy Dispersive X-ray spectra for the AgNPs synthesized using
B. amyloliquefaciens (B. a.), and
B. subtillis (B. s.).

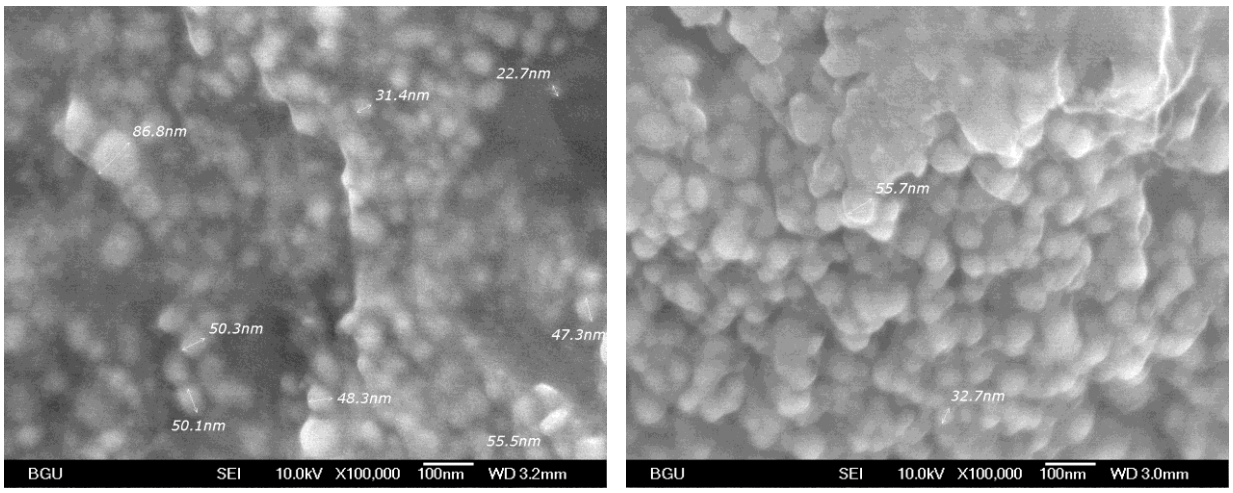



Fig. 4.7 Silver nanoparticles biosynthesized in the presence of *Bacillus amyloliquefaciens* bacterial supernatant and *Bacillus subtillis*

### 4.3.3 Structural investigations by X-ray diffraction analysis

The crystalline nature of silver nanoparticles was analyzed by XRD using a Philips PW 1050/70 X-ray powder diffractometer with graphite monochromator using CuK$\alpha$1 ($\lambda$ =1,54Å), at a voltage of 40 kV, a current of 28 mA, in the scan range 20-60 °, in Bragg-Brentano geometry.

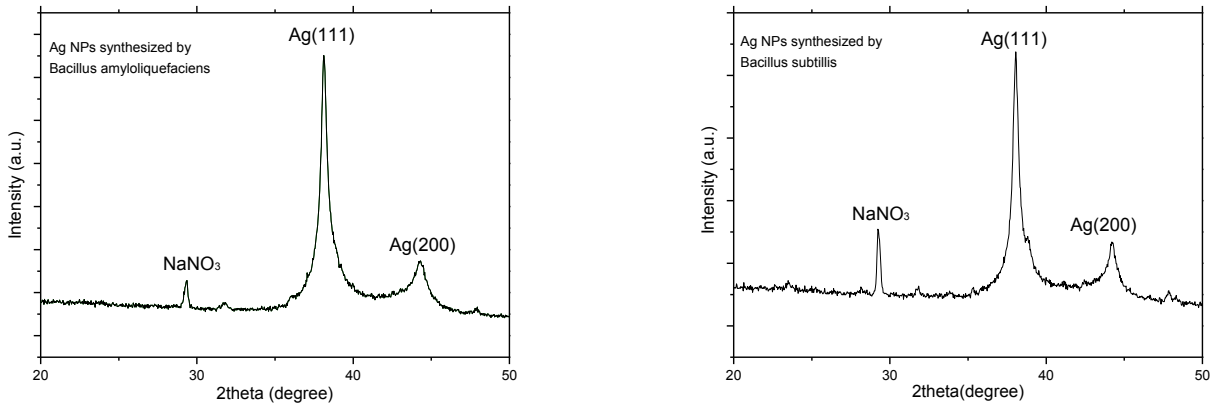



Fig.4.9 Diffractograms of biosynthesized silver nanoparticles

X-ray diffraction was performed in order to obtain information about the structure of the crystalline material. On the spectrum ranging between $2\theta = 10° \div 60°$ (Fig. 4.9.), 2 peaks for each sample can be observed, at $2\theta = 38.09°$, and $2\theta = 44.25°$, which coincide with the (111) and (200) diffraction planes for face-centered cubic silver (00-004-0783). The third peak present in both diffractograms, at $2\theta = 29.28°$ corresponds to sodium nitrate (00-079-2056). The reasons for the presence of this compound as well as ways to eliminate it were mentioned previously. After deconvolution of the 2 diffraction patterns with a Pearson7 function, the exact peak position, intensity and FWHM have been obtained. Using the Debye-Scherrer equation, the crystallite size was calculated, for all the diffraction peaks corresponding to silver. The silver nanoparticles synthesized using B.

amyloliquefaciens 1853, for the (111) orientation have a mean crystallite size of 10.93 nm, while for the (200) orientation the mean crystallite size is 6.55 nm. The samples obtained with B. subtillis 10833 exhibit slightly larger crystallite sizes of 23.11 nm and 9.53 nm, for the (111) and (200) peaks, respectively.

### 4.3.4 Size distribution of obtained nanoparticles

The nanoparticle size distribution was investigated by Dynamic Light Scattering (DLS) in aqueous samples. A CGS-3 Compact Goniometer and LSE-5003 correlator were used. The results were obtained after 15 cycles at a 90 ° angle and 26 °C.

The DLS graph of colloid silver nanoparticles obtained using B. amyloliquefaciens (figure 4.10.a) shows that the colloid includes 2 populations of nanoparticles. The mean size of the first population is 0.506 nm/radius and for the second one is around 36.93 nm/radius, supporting the results obtained from SEM analyses. The AgNPs obtained using B. subtillis (figure 4.10.b) show a mean size of each population of 0.918, 5.433 and 67.89 nm/radius. Besides supporting the SEM observations, the DLS results show that even smaller particles can be synthesized by this method.

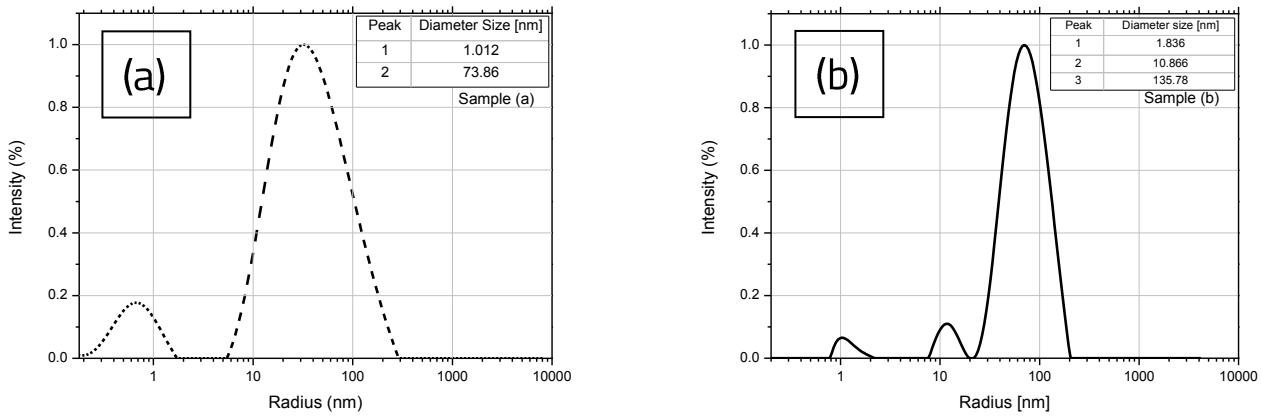



Fig.4.10. DLS results for particle size distribution of AgNPs,
synthesized by green chemistry using the supernatant from
(a) Bacillus amyloliquefaciens
(b) Bacillus subtilis

## 4.4. Antimicrobial activity of AgNPs

Five microbial strains and one fungus strain were selected for antimicrobial activity studies. The strains were grown on MacConkey and Sabouraud agar for 24 hours in order to obtain fresh cultures. After this, several colonies of each culture were suspended in 3ml NaCl and used for further tests. The solid medium used for antimicrobial activity was composed of 1 g/l yeast extract; 18 g/l agar-agar; 5 g/l sodium nitrate; 0.2 g/l glucose. The silver nanoparticles activity was assessed against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella, Streptococcus pyogenes and Candida albicans. The potential of silver nanoparticles regarding the antimicrobial activity was determined using the disk diffusion method (as described in CLSI Clinical and Laboratory Standards Institute: M02-A11 standard (Performance Standards for Antimicrobial Disk Susceptibility Tests)), by impregnating 15μl AgNP-containing solution on each 6-mm diameter disk made of glass microfiber filters from WhatmanTM. Furthermore, the synergistic effect of silver nanoparticles in combination with different antibiotics against the microorganisms was investigated, using 6 mm disks containing Ciprofloxacin 5 μg for bacteria and Fluconazole 25μg for fungi, impregnated with 15μl AgNP-containing solution. Disks containing Ciprofloxacin 5 μg and Fluconazole 25μg were used as control. The disks were placed on the surface of the culture containing petri dishes. After 24 h of incubation at 35 °C, the inhibition diameter zone was measured and compared to the controls. A series of three antibacterial activity tests were performed, the inhibition zone diameter was measured with digital calipers and the standard deviation was calculated.
The results of antibacterial and antifungal activities of both solutions containing silver nanoparticles against five bacterial strains and one fungus strain, using the disk diffusion method, can be observed in figures 4.12, 4.13 and 4.14.. Figure 4.12. shows the variation in inhibition zone diameter, for the disks containing AgNPs, the control antibiotic disks, and AgNPs in conjunction with antibiotics.

Although there is a clear difference between the activity of the AgNPs infused disks and the control samples in the case of the 5 bacteria strains, the antimicrobial activity in the case of C. albicans, is noticeably higher compared to the control antibiotic. The Fluconazole disks exhibited little to no activity on the C. albicans strain, only in conjunction with AgNPs the inhibition zone was measurable. The beneficial effect of AgNPs concerning the antibiotic activity was calculated as the percentage fold increase in antibacterial effect, shown in figure 4.13.[75].

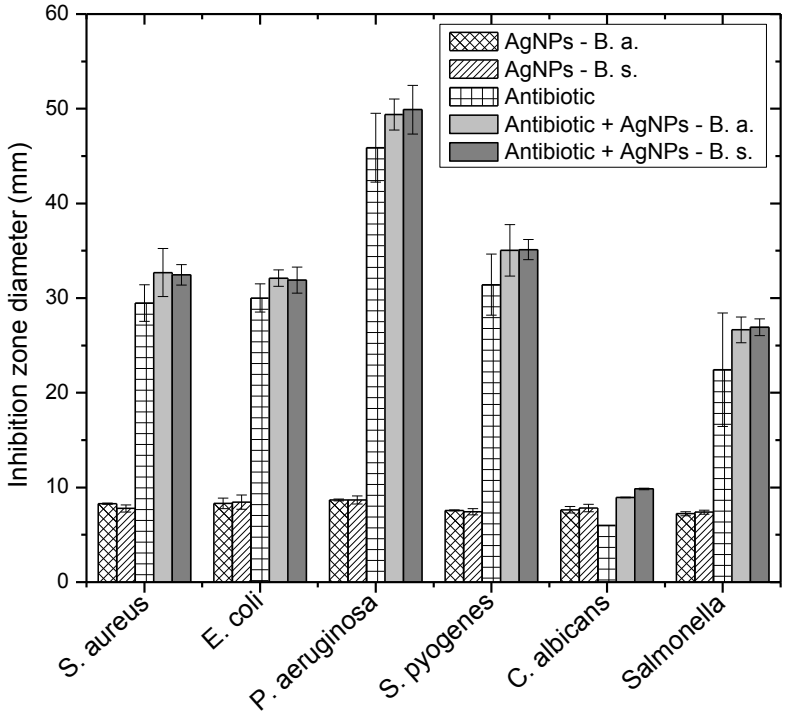



Figura 4.12 Inhibition diameter variation for the tested strains, for the AgNPs disks, the antibiotic disks and the synergistic effect of the AgNPs + antibiotic combination.

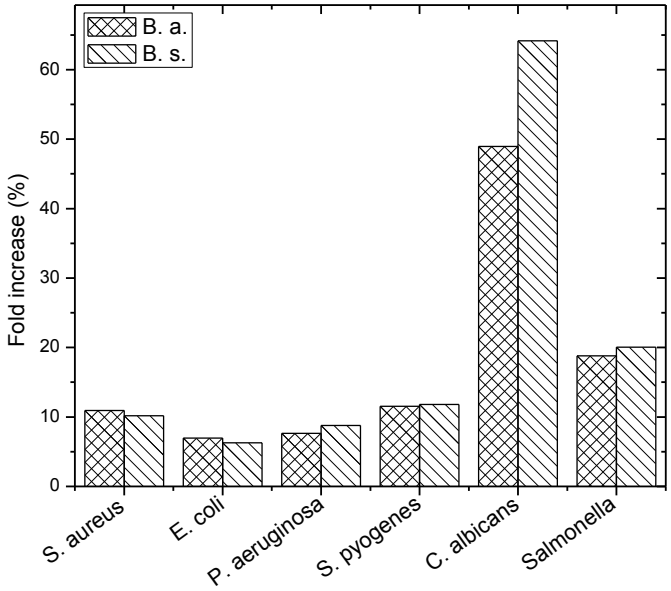



Figura 4.13 Percentage fold increase in antibacterial effect of antibiotics with AgNPs against test strains, calculated with equation ((b-a)/a) ×100 (%), where b represents the inhibition zone diameter for the AgNPs + antibiotic combination, and a represents the inhibition zone diameter for the antibiotic disk[75], [65],

While using different species of microorganisms for nanoparticles synthesis the results can vary in regard to their size and shape. This observation is relevant even when using the same species, but

different parameters of the process, like pH of the precursors, incubation time, precursor concentration.

It was reported that AgNPs get attached to the cell membrane of the bacterium and also penetrate inside the bacteria. The bacterial membrane contains sulfur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA.

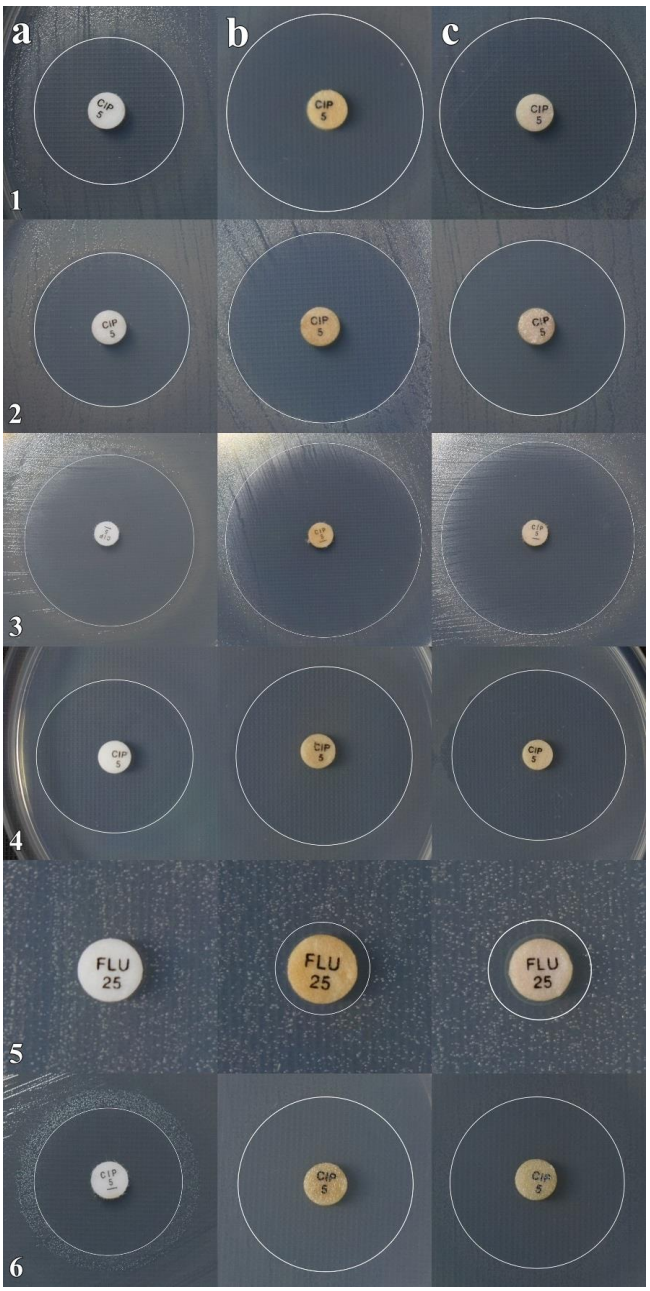



Fig. 4.14 Photographs of the inhibition zone of *S. aureus* (1), *E. coli* (2), *P. aeruginosa* (3), *S. pyogenes* (4), *C. albicans* (5) and *Salmonella* (6) cultures: a – antibiotic, b – antibiotic + B.s. AgNPs, c – antibiotic + B.a. AgNPs [75]

## 4.5 Conclusions

- B. amyloliquefaciens 1853 and B. subtillis 10833 present the ability to synthesize extracellularly silver nanoparticles by bioreduction from 1 mM $AgNO_3$ precursor solution. During UV-visible analysis, peaks for both colloids at 418 nm, 414 nm, respectively, were observed.
- SEM analysis showed the spherical shape of nanoparticles with size less than 100 nm. X-ray diffraction analysis confirmed that the highest peak of silver crystal corresponds to the (111) diffraction plane. DLS size distribution confirms the SEM results in regard to nanoparticle size.
- The AgNPs synthesized using *B. amyloliquefaciens* 1853 and *B. subtillis 10833* have presented significant antibacterial and antifungal activity, either standalone, or in conjunction with antibiotics.
- The advantage of the proposed synthesis method consists in its economic efficiency (the wide availability of the B. amyloliquefaciens and B. subtilis strains, and the one-step synthesis approach, consisting in reduction of silver ions by the bacteria-free enzymatic extract).
- Potential drawbacks of our method may include the high synthesis duration (48 hours), reproducibility issues (due to different behavior in bacterial strain enzymatic activities, the presence of trace impurities which may inhibit bacteria activity, the careful maintaining of identical synthesis conditions). Through the proposed method, number concentrations of silver nanoparticles in the range of $1\text{-}7{\cdot}10^{11}$ have been obtained at mass concentrations of 0.095 mg/L, similar to other commercial silver nanoparticle products.
- Further research in this domain will be focused on perfecting the synthesis duration by using other bacterial strains and in assessing the long-term stability of the colloidal solutions in different environments, which is of upmost importance for practical applications. The proposed synthesis approach shows promising potential in the optical domain (refraction index of material/ color tuning by nanoparticles incorporation), in biochemistry applications (biosensor assays, biological tags), as well as in electrothermal conductive inks and paints manufacture.

# CHAPTER V
# RESEARCHES ON BIOSYNTHESIS AND CHARACTERIZATION OF SILVER CHLORIDE NANOPARTICLES

## 5.1 Contributions to the study of AgCl nanoparticles

In this chapter, I have presented the results of the researches on biosynthesis of silver chloride nanoparticles. These were synthesized using *Rhodotorula Mucilaginosa, Enterobacter Faecalis, Pantoea* and *Raoultella Planticola* microorganisms and aqueous AgNO3 as a precursor.

The plasmonic resonance of nanoparticle-containing solution showed a UV absorption maximum at about 440 nm by UV-vis spectrophotometry (UV-vis). Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Dispersive Energy Spectroscopy (EDX) and X-ray Diffraction (XRD), Atomic Force Microscopy (AFM), and Selected Area Electron Spectroscopy (SAED) the presence of spherical silver chloride nanoparticles with a centered cubic crystal structure and a mean particle size of approximately 10-50 nm [76], [74].

It has been demonstrated that silver chloride nanoparticles have the ability to inhibit the growth of various microorganisms, including bacteria and fungi, which make them suitable for antimicrobial applications [80].

In this study we have shown that silver chloride nanoparticles biosynthesized have the ability to inhibit the growth of various microorganisms such as, *Staphylococcus aureus, Streptococcus Pyogenes, Salmonella* or *Bacillus amiloliquefaciens* [80]

## 5.2 Silver chloride nanoparticles synthesis

### 5.2.1 Microorganisms and culture conditions

*Rhodotorula Mucilaginosa, Enterobacter Faecalis, Pantoea* and *Raoultella Planticola* used in this study were provided by Soroka University Medical Center in Beersheva, Israel. The microorganisms were cultured in solid medium, Sabouraud agar supplied by Scharlau Chemicals and incubated at 35 ° C for 48 hours.

### 5.2.2 Biosynthesis of AgCl nanoparticles using *Rhodotorula Mucilaginosa, Enterobacter Faecalis, Pantoea* and *Raoultella Planticola*

To synthesize silver chloride nanoparticles, 1 µl of microbial strains were inoculated into test tubes containing 15 ml of growth medium, Sigma Aldrich Infusion Brain-Cord agar. The liquid media contained beef heart (infusion from 250 g), 5 g / l; calf viruses (infusion from 200 g), 12.5 g / l; disodium hydrogen phosphate, 2.5 g / l; D (+) - glucose, 2 g / l; peptone, 10 g / l; Sodium Chloride 5 g /

L The liquid culture was kept in the thermostat at 35 ° C for 24 hours followed by centrifugation at 4000 rpm for 30 minutes.

Supernatant and biomass were tested in parallel. In the first situation, 5 ml of the supernatant was used, while for the second set the biomass was maintained with the addition of 5 ml of distilled water. Culture, supernatant and biomass + distilled water and precursors were kept for control. Samples were kept in a thermostat set at 35 ° C for 48 hours.

After incubating in the thermostat for 48 hours at 35 ° C, the final color of colloids containing the biomass of the microorganisms changed from light yellow to brown.

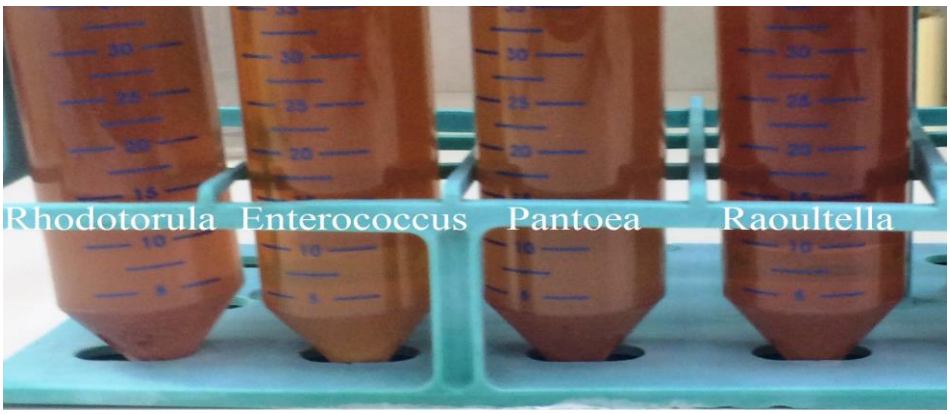



Fig. 5.2. Colloids with byosynthesized AgCl nanoparticles

Color change is an indication for the formation of nanoparticles. Figure 5.2 shows the difference in brown tones depending on microorganisms used in biosynthesis processes. Control samples remained unchanged.

## 5.3 The characterization of the obtained structure and the interpretation of found data. Used equipments.

### 5.3.1 Surface plasmon resonance

In view of the optical indication (color change), the UV-visible absorption spectra of the colloidal nanoparticle solutions (shown in Figure 5.3) were measured. The ultraviolet-visible spectral analysis was performed using the Jasco V-630 spectrophotometer. The spectra were measured in the range 200-600 nm with a wavelength of 1.5 nm.

Figures 5.3 and 5.4 respectively. correspond to the visible UV absorption spectra of solutions containing AgCl NPs synthesized using the microorganisms presented in 5.1. Of these, the spectra specific to nanoparticles analysis synthesized in the presence of *Raoultella Planticola* and *Rhodotorula Mucilaginosa* showed peak absorption peaks around 440 nm, specific for AgCl NPs. *Enterobacter Faecalis* and *Pantoea* spectra are much broader and undefined. It is believed that these bacteria have the ability to reduce Ag + ions, but it is the problem of a polydispersed colloid.

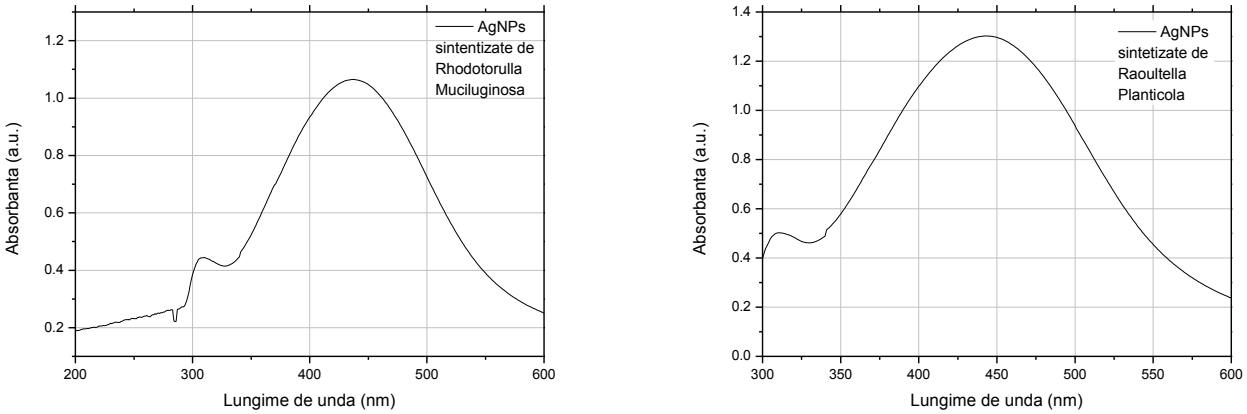



Fig. 5.3 UV-visible spectra of colloids with AgCl nanoparticles, biosynthesized by *Rhodotorula Mucilaginosa*, respectively *Raoultella Planticola*

Several articles have provided similar results in terms of absorption spectrum. The reports confirmed that peaks of approximately 440 nm coincided with the plasmon resonance of silver chloride nanoparticles [4], [5].

## 5.3.2 Structural investigations through analysis using X-ray diffraction

The crystalline nature of the silver chloride nanoparticles was analyzed by XRD using a Philips PW 1050/70 powder diffractometer with graphite monochromate using CuK$\alpha$1 ($\lambda$ = 1.54Å) at a voltage of 40 kV, a current of 28 mA, in the scanning range 10 ÷ 80 °, in the Bragg-Brentano geometry.

The crystalline structure of the synthesized nanoparticles obtained by X-ray diffraction is confirmed by the diffraction peaks shown in Figure 5.5, which correspond to the planes (111), (200) and (220), (311), (222), (440) specific to the cubic structure with centered faces of the AgCl crystal [11].

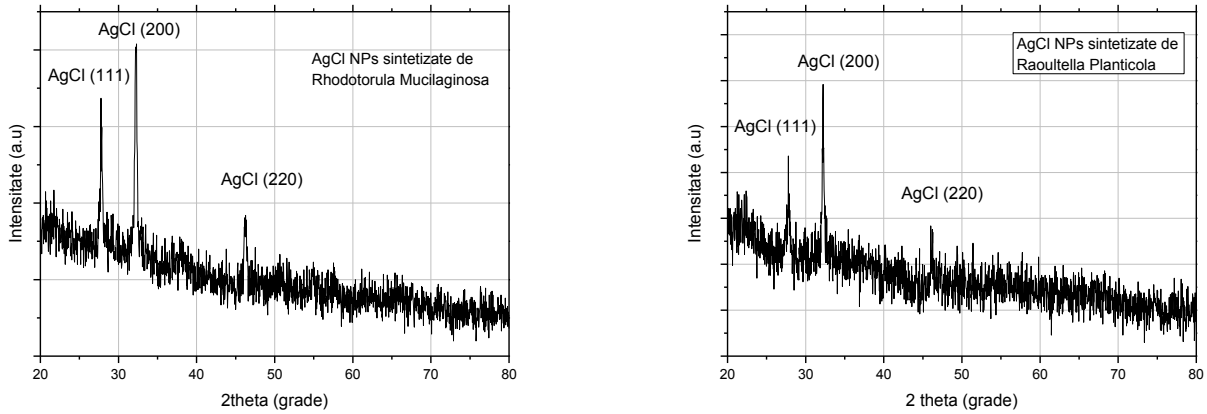



Fig. 5.5 X-ray diffraction spectra for AgCl nanoparticles *Rhodotorula Mucilaginosa*, respectively *Raoultella Planticola*

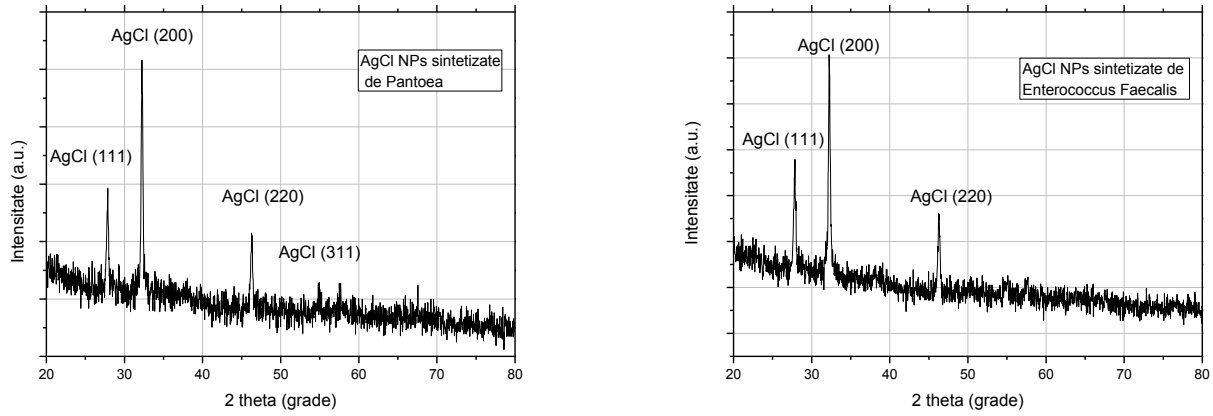



Fig. 5.6 X-ray diffraction spectra for AgCl nanoparticlessynthesized by the *Pantoea*, respectively *Enterococcus Faecalis*

### 5.3.5 Structural characterization of nanoparticles using Transmission Electron Microscopy (TEM) and Selected Area Electron Diffraction (SAED)

TEM and SAED analyzes were performed with a Tecnai 12 Twin (FEI) microscope operating at 100kV. This microscope is equipped with a fully computerized goniometer (with ± 60 degrees of elasticity inclination) and two CCD MultiScan cameras (Gatan 791 and wide angle Gatan 794) allowing high quality images to be recorded (Figure 5.x (A)).

Sample Preparation: A drop of colloidal solution containing silver chloride nanoparticles was placed on a 3 mm carbon-coated copper screen, special for TEM analyzes. The samples were then allowed to evaporate (Figure 5.7 (B)). Surface areas were previously cut using a plasma-cleaner from Harrick Plasma.

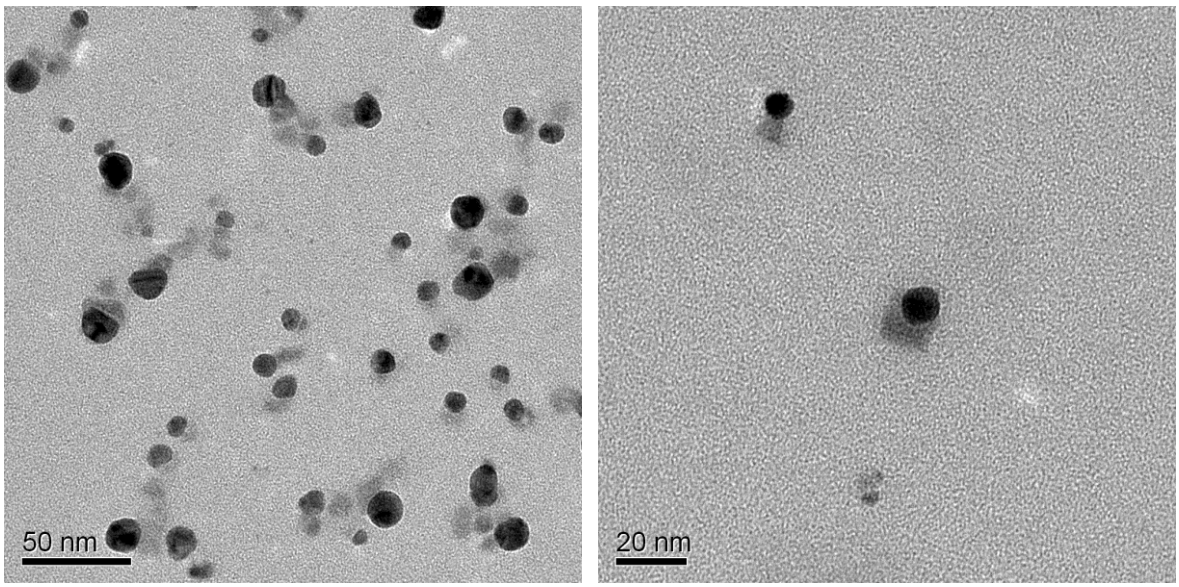



Fig. 5.8 TEM images of nanoparticles synthesized by *Rhodotorulla Mucilaginosa*

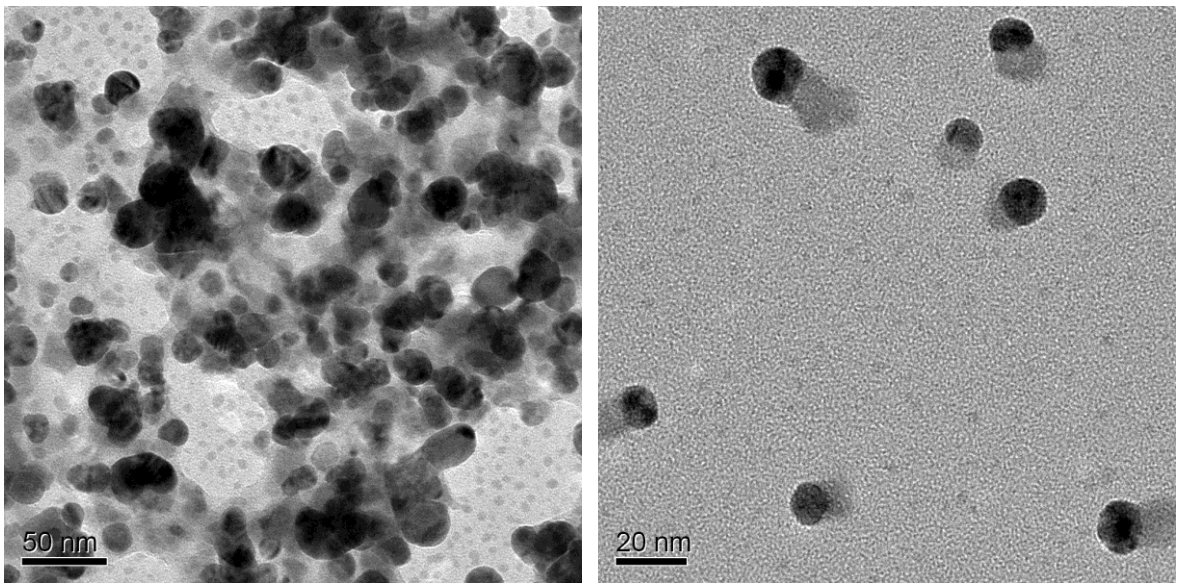



Fig. 5.9 TEM images of nanoparticles synthesized by *Raoultella Planticola*

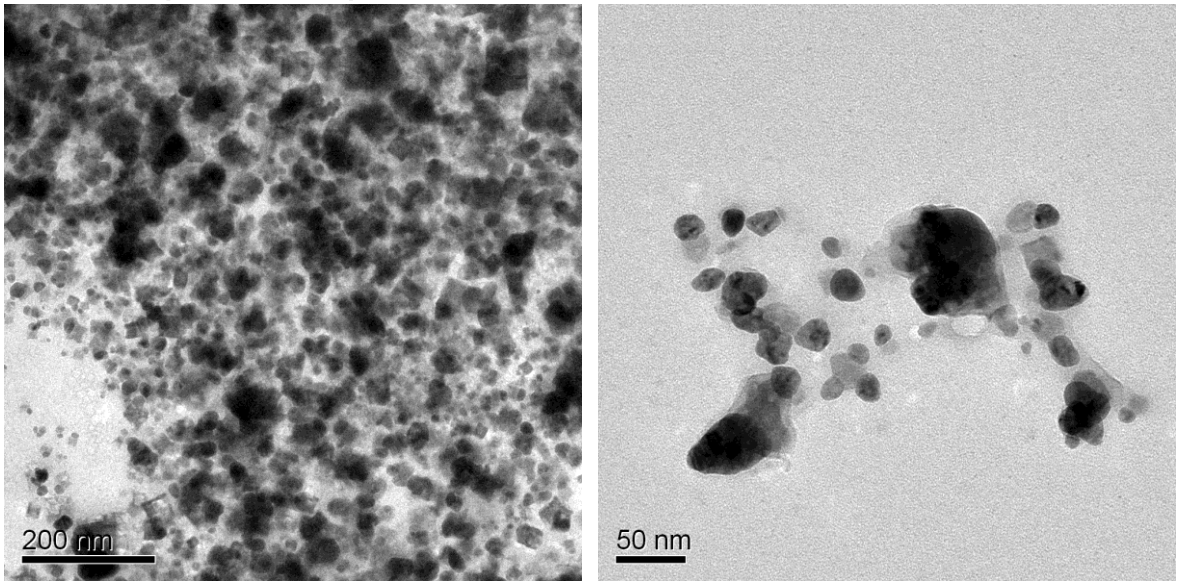



Fig. 5.10 TEM images of nanoparticles synthesized by *Pantoea*

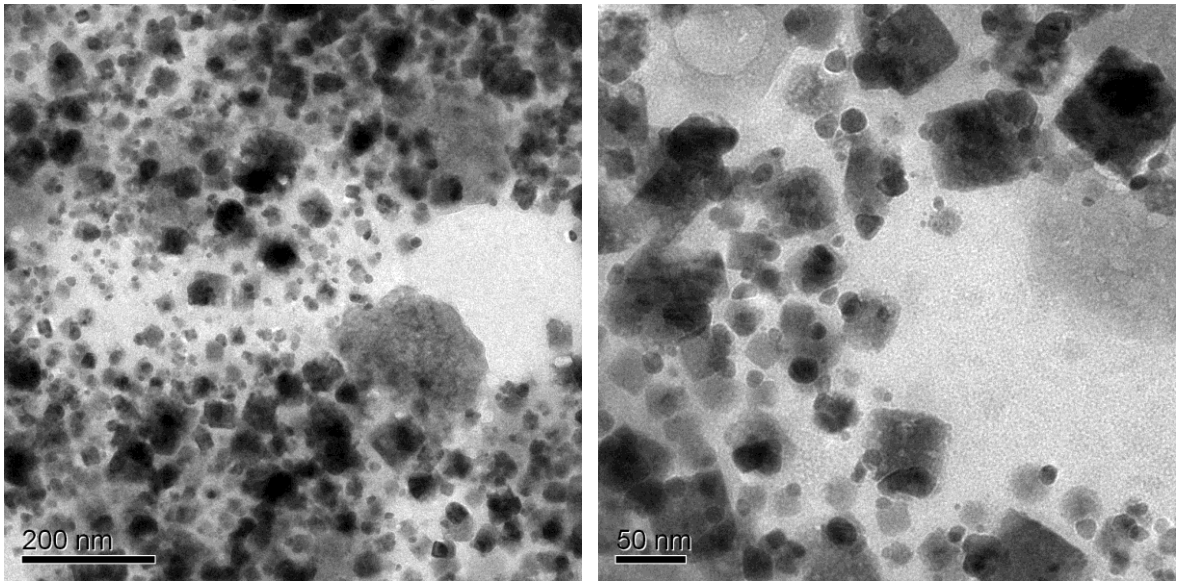



Fig. 5.11 TEM images of nanoparticles synthesized by *Enterococcus Faecalis*

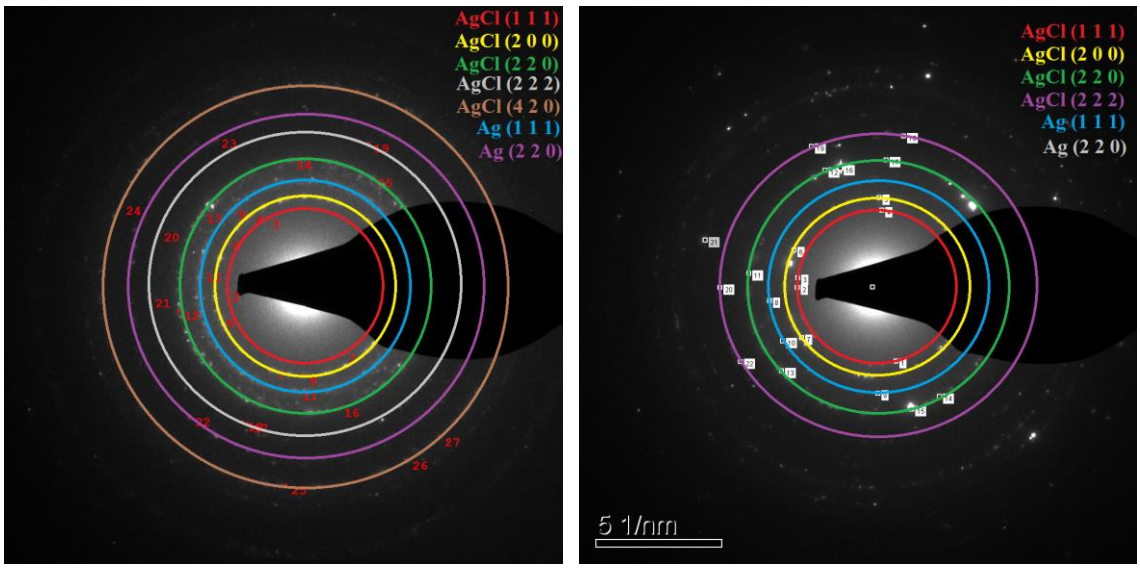



Fig. 5.12 The electron diffraction patterns in the selected area of the AgCl or Ag nanoparticles synthesized in the presence of *Rhodotorula Mucilaginosa* (a) and *Raoultella Planticola* (b)

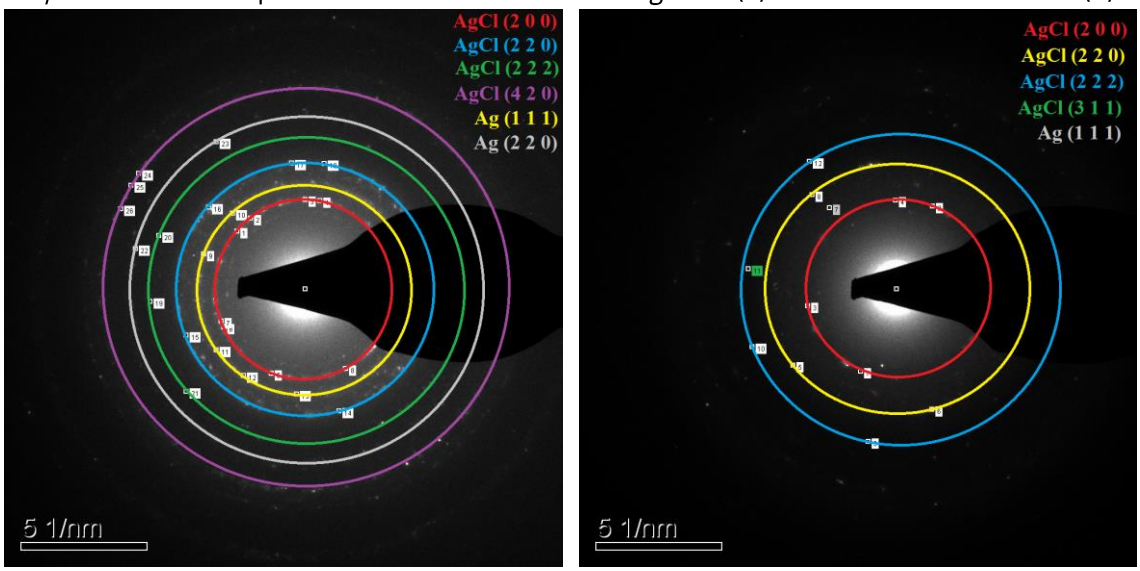



Fig. 5.13 The electron diffraction patterns in the selected area of the AgCl and Ag nanoparticles synthesized in the presence of the microorganisms *Pantoea* (a) și *Enterococcus Faecalis* (b)

Silver chloride nanoparticles synthesized using *Rhodotorulla Muciluginosa* are approximately 15 nm (Figure 5.8 - a, 5.8 - b), according to TEM analysis. Their monodispersity leads to a differentiation of the results from other microorganisms. AgCl NPs synthesized in the presence of Raoutella Planticola also have very small dimensions in the 10-30 nm range (5.9-a, 5.9-b).

The *Pantoea* and *Enterococcus faecalis* mediated the synthesis of silver chloride nanoparticles in a much wider range, their dimensions reaching up to 50 nm (Figure 5.10.a, 5.10.b, 5.11-a, 5.11-b.) Spherical shape of nanoparticles can be seen from images obtained at TEM.

Figures 5.12 and 5.13 respectively shows the electron diffraction patterns of the selected area for the AgCl and Ag crystals of microorganism-mediated green synthesis samples. The diffraction patterns confirm the results obtained by the XRD analysis presented in 5.1. Concentric rings indicate that nanoparticles are crystalline. Also, the interplanar distances present in the diffraction patterns

correspond to the indexing of cubic structures with centered faces of silver and silver chloride crystals.

It can be seen that the predominant planes are (1 1 1), (2 0 0), (2 2 0), (3 1 1), (2 2 2), (4 0 0) and (4 2 0) of AgCl that show more (bright) points in their structure. Plans corresponding to the Ag crystals most commonly found in the nanoparticles synthesized using microganisms are (111) and (220) respectively.

Increasing the sodium chloride concentration (or other precursor used for the synthesis of Cl-based nanoparticles) could diminish the presence of silver crystals, forming the desired AgCl configuration.

### 5.3.4 Structural and Morphological Analysis of Particles by Scanning Electron Microscopy. Determination of chemical composition by X-ray microanalysis

An scanning electron microscope JSM 7400f (SEM) with an energy dispersion spectroscopy (EDS) platform (Figure 5.14) was used for the morphological characteristics and chemical composition. The silver chloride nanoparticle colloid placed on the copper screen from the TEM analyzes was sprayed with a thin layer of platinum (Figure 5.14 (A) to ensure better conductivity. Afterwards the samples were mounted on a double- (figure 5.14 (B)) The acceleration voltage was fixed at 10 kV.

The platinum layer deposited on samples prepared and used for TEM analysis for SEM analysis consisted of 2 spraying sessions of 10 seconds.

SEM analyzes (Figures 5.15, 5.16, 5.17, 5.18) confirmed the presence of nano-scaled particles and showed their spherical shape. As can be seen on SEM micrographs, silver chloride nanoparticles range from 10-50 nm. Particle size / antimicrobial efficacy report was previously reported, smaller particles, due to their much larger area, are expected to exhibit much greater antibacterial efficacy

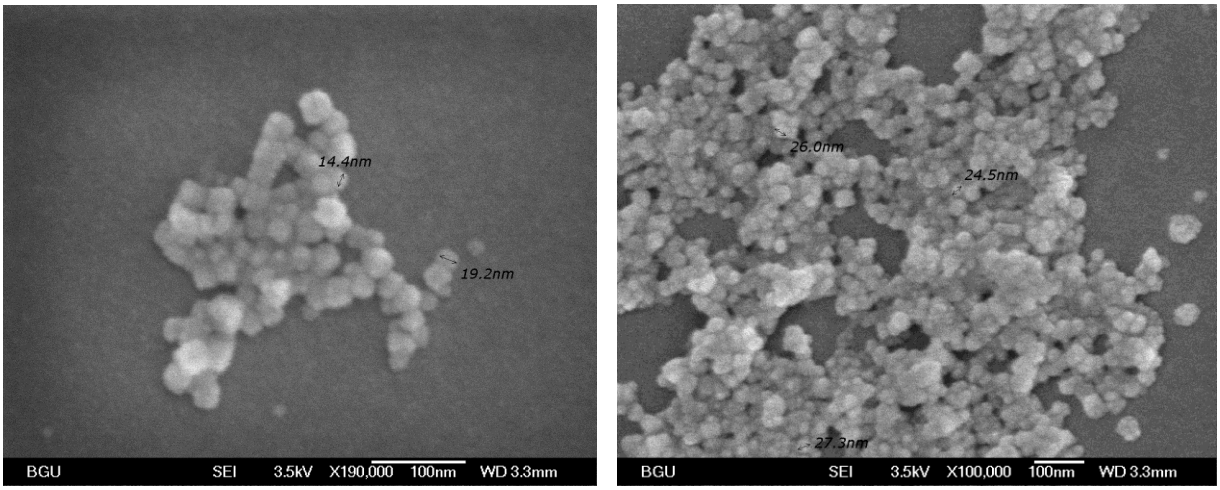



Fig. 5.15 SEM images of nanoparticles synthesized by *Rhodotorulla Mucilaginosa* and Raoultella Planticola

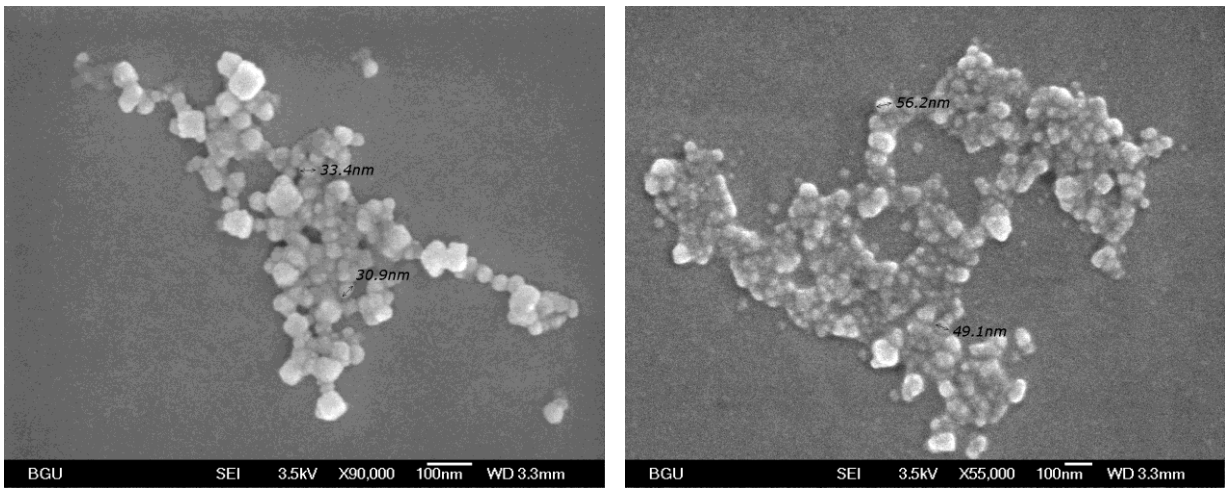



Fig. 5.17 SEM images of nanoparticles synthesized by *Pantoea* and *Enterococcus faecalis*

The EDS spectra presented in Figures 5.19 and 5.20 show the presence of the main elements, namely Ag and Cl. EDS analysis also revealed other elements that can be found on samples due to the preparation steps (copper grid, carbon band, platinum thin film).

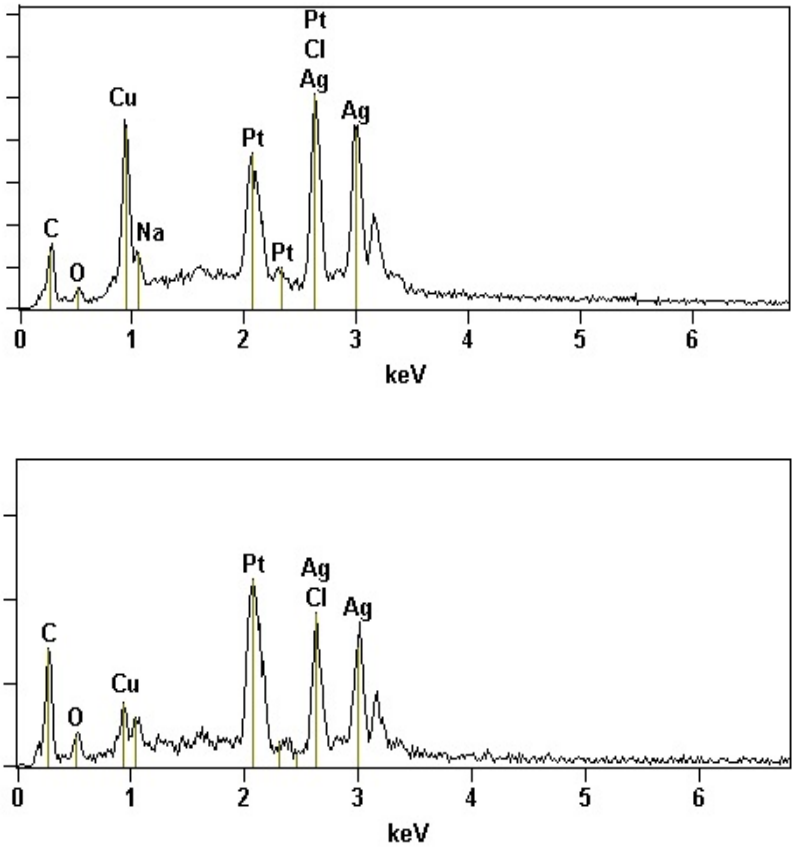
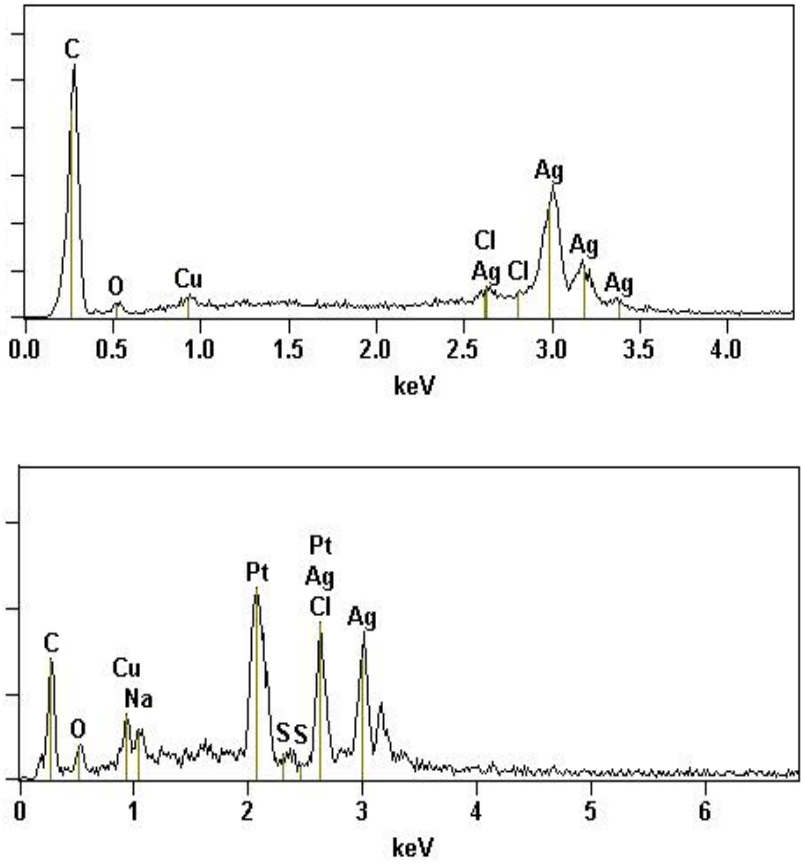



Fig.5.19 EDS spectra of AgCl NPs biosynthesized in the presence Rhodotorulla Mucilaginosa, respectively, Raoutella Planticola




Fig, 5.20 EDS spectra of AgCl NPs biosynthesized in the presence *Pantoea*, respectively, *Enterococcus Faecalis*

An explanation for the presence of silver chloride nanoparticles can be based on the interaction between silver nitrate and bacteria, which was previously cultivated in the Brain Heart Infusion containing sodium chloride.

### 5.3.5 Average nanoparticles diameter determined from AFM cross-section

To obtain additional information on the morphology and size of biosynthetic nanoparticles, we used an MFP-3D-BIO atomic force microscope. It offers a very high sensitivity and accurate images and measurements possible on an inverted optical platform. Closed-loop nanoparticle sensors on all three axes provide images without distortion on samples as small as proteins and as large as cells - both in air and in liquid.

Samples of silver chloride nanoparticles reduced by Rhodotorula mucilaginosa, Raoultella Planticola, Pantoea and Enterococcus faecalis were centrifuged at 2000 rpm for 10 minutes and the pellet obtained was washed with deionized water to remove any possible biomass. The pellet was redispersed in a small amount of deionized water by ultrasonication and used to drip onto a Si

substrate, then allowed to dry. The samples were analyzed using the AFM non-contact mode. Height data was collected at a scanning frequency of 2.4 Hz.

The biosynthetic silver nanoparticles were scanned using AFM to understand the exact configuration of the biosynthesized nanoparticles, in particular to verify that silver nanoparticles are more or less homogeneous in size and have spherical shape.

Figures 5.21 show an AFM image representative of silver chloride nanoparticles synthesized in the presence of microorganisms. For biosynthesized nanoparticles in the presence of Rhodotorula Mucilaginosa, Raoultella Planticola, spherical nanoparticles can be observed in the 5-25 nm domains. For biosynthesized nanoparticles in the presence of Pantoea and Enterococcus Faecalis, a number of overlapping spherical nanoparticles can be observed, the spherical shape of which is still visible, and the dimensions are somewhat larger at about 40-60 nm. AgClNPs are presented both in perspective and top view. 3D information is embedded in both views. In perspective, the 3D nature of the image is obvious. In the top view, the intensity of the color reflects the height of nanoparticles.

For cross sections, nanoparticle sizes are rendered on the Z axis (dimensions not affected as they are performed in non-contact mode), the X axis representing the scanning distance of the sample.

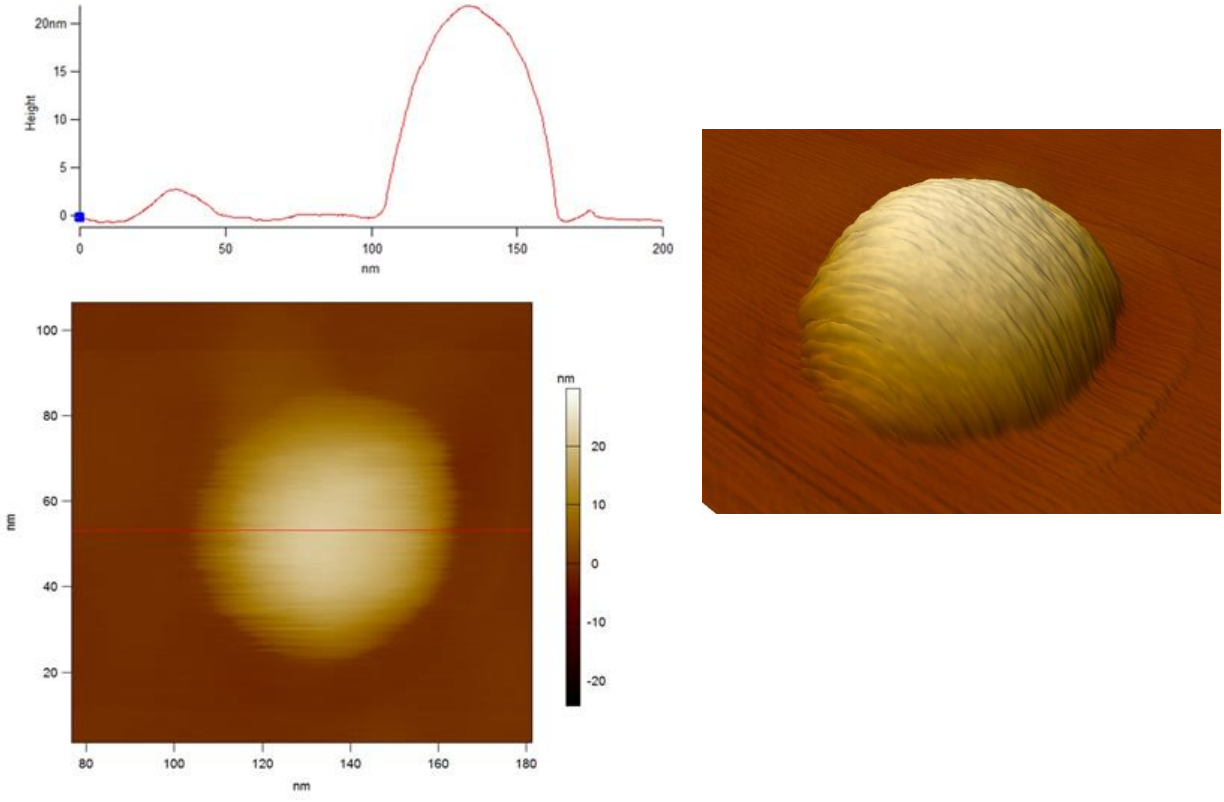



Fig. 5.21 Cross section, respectively, 3D representation of AgCl NPs biosynthesized in the presence of *Rhodotorulla Mucilaginosa*

## 5.4 Antimicrobial activity of silver chloride nanoparticles

Four bacterial strains were selected for studies of antimicrobial activity. The strains used for applicability testing were grown in solid medium consisting of yeast extract of 1 g / l; 18 g / l agar-agar; 5 g / l sodium nitrate; 0.2 g / l glucose. Several colonies of each culture were suspended in 3 ml NaCl and used for additional assays.

The activity of silver chloride nanoparticles was evaluated against *Staphylococcus aureus, Streptococcus pyogenes, Salmonella, Bacillus amyoliquefaciens*. The potential of silver chloride nanoparticles for antimicrobial activity was determined using the disc diffusion method (as described in the CLSI Clinical and Laboratory Standards Institute: M02-A11 standard, by impregnating 15 μl of solution containing AgCl NPs on each 6 mm diameter disc made from WhatmanTM glass microfibre filters. Furthermore, the synergistic effect of silver chloride nanoparticles in combination with different antibiotics against microorganisms was investigated using 6 mm disks containing Ciprofloxacin 5 μg (CIP 5), Vancomycin 30 μg (VA 30), Erythromycin 15 μg (E 15) , impregnated with a solution of 15 μl of AgCl NPs.

Discs containing Ciprofloxacin 5 μg, Vancomycin 30 μg, Erythromycin 15 μg were used for control. The disks were placed on the surface of the culture contained in the Petri plates. After 24 hours of incubation at 33 ° C, the inhibition diameter zone was measured and compared with the control samples. A series of three antibacterial activity tests were performed and the diameter of the inhibition zone was measured with a digital caliper and the standard deviation was calculated.

The results of antibacterial and antifungal activities of both solutions containing silver nanoparticles against four bacterial strains using the disc diffusion method can be seen in Figures 5.25, 5.26 and 5.27. Figure 5.25 shows the variation of the inhibition zone diameter for discs containing AgCl NPs, control antibiotic discs and AgCl NPs together with antibiotics. It can be observed that two types of antibiotics (Ciprofloxacin 5 μg, Erythromycin 15 μg) were used for the strain of *Staphylococcus aureus* to determine the synergism between these and AgCl NPs. Also two types of antibiotics were used for Steptococcus pyogenes, this time, Vancomycin 30 μg, Erythromycin 15 μg. For *Samonella* and *Bacillus amyloliquefaciens*, synergism was determined using only Ciprofloxacin 5 μg.

The antimicrobial activity of the impregnated discs only with solutions containing silver chloride nanoparticles developed significant antimicrobial activity for those synthesized in the presence of the *Raoultella Planticola* bacteria (solution b). Synergism between antibiotics and nanoparticles has shown important results when using Erithromycin in combination with AgCl NPs synthesized by *Rhodotorulla Mucilaginosa*. Activity was determined against Staphylococcus aureus.

The bio-reduced silver chloride nanoparticles have been found to be active in stopping and inhibiting the growth of *S. aureus, S. pyogenes, Salmonella* and *Bacillus amylolyquefaciens*.

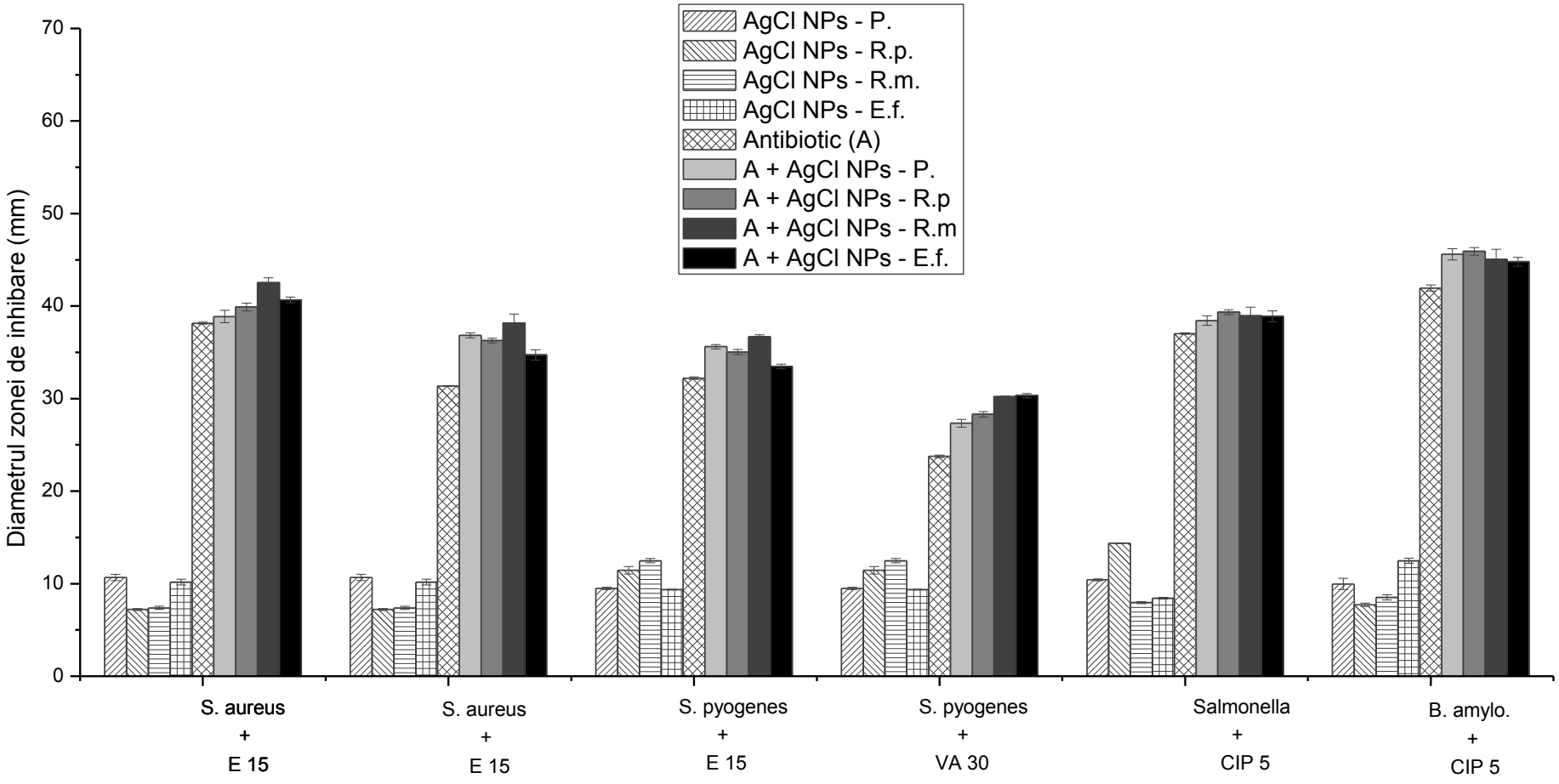



Fig. 5.25 Figure 5.25 The variation of the inhibition diameter for the strains tested, for AgCl NPs disks, antibiotic disks and the effect of synergism between AgCl NPs + antibiotics.

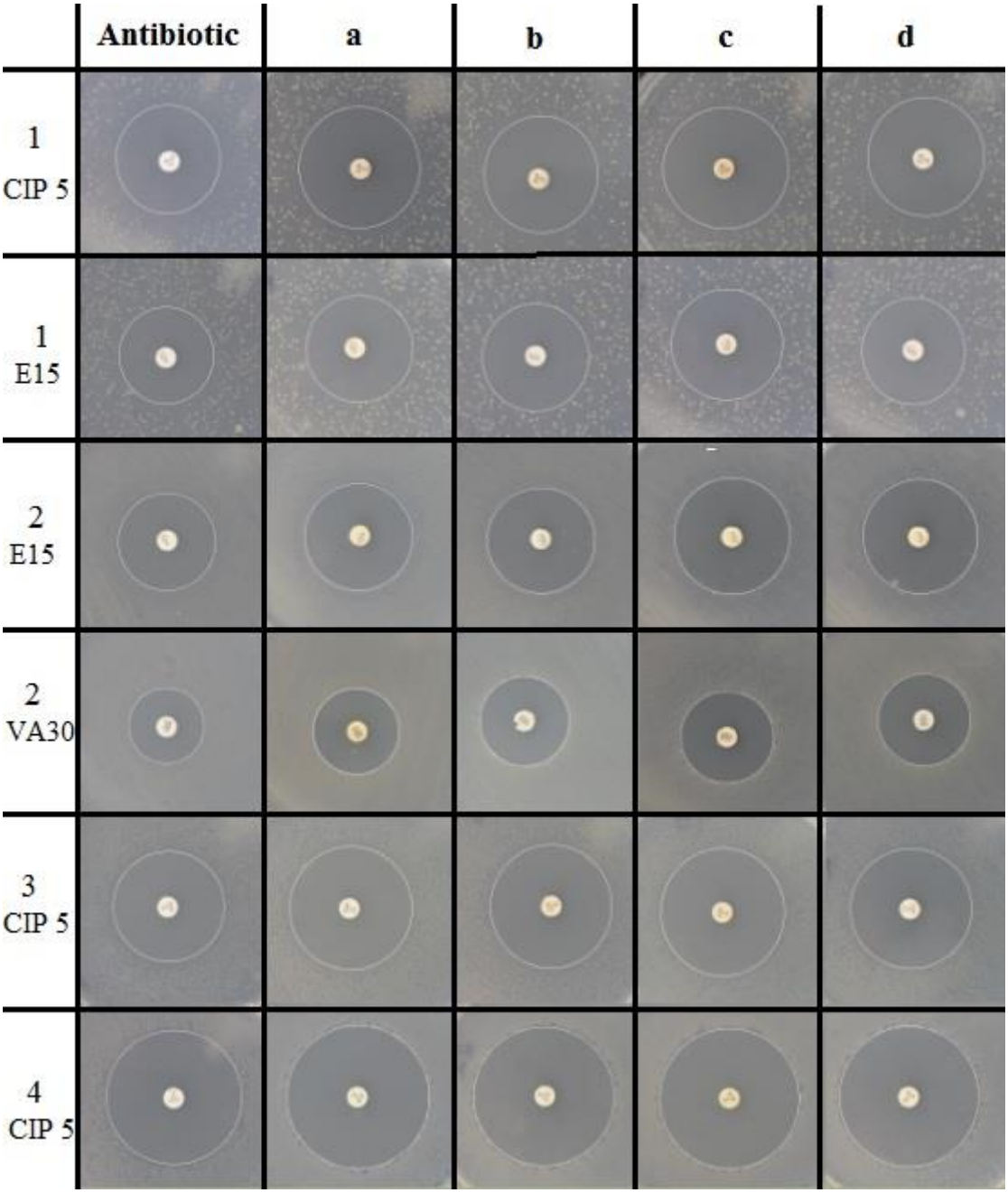



Fig. 5.26. Images of the inhibition zone of (1) Staphylococcus aureus, (2) Streptococcus pyogenes, (3) Salmonella, (4) Bacillus amyloliquefaciens, in the presence of antibiotics Erythromycin (E15), Ciprofloxacin (CIP 15) and Vancomicin (VA 30), respectively.

Synergism developed between antibiotic and silver chloride nanoparticles.
a - AgCl NPs synthesized in the presence of Pantoea
b - AgCl NPs synthesized in the presence of Raoultella Planticola
c-AgCl NPs synthesized in the presence of Rhodotorula Mucilaginosa
d - AgCl NPs synthesized in the presence of Enterococcus faecalis

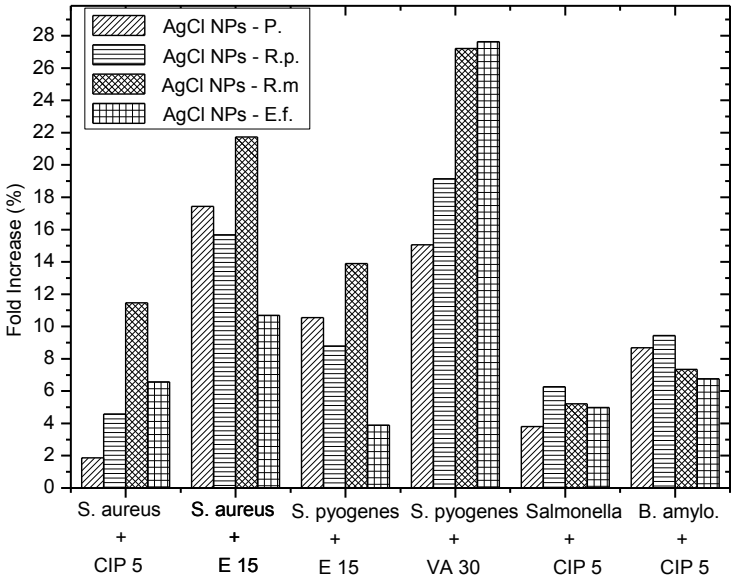



Figure 5.27. Percentage increase in the antibacterial effect of antibiotics with AgCl NPs against the test strains, calculated with the equation ((ba) / a) x 100 (%), where b represents the diameter of the inhibition zone for the combination of AgNPs + antibiotic and represents the diameter of the inhibition zone for the antibiotic
disc

## 5.5 Conclusions

- Silver chloride nanoparticles were synthesized by bio-reduction. It has been shown that the aqueous enzymatic extracts of Rhodotorula *Mucilaginosa, Enterobacter Faecalis, Pantoea* and *Raoultella Planticola,* are capable of synthesizing silver chloride nanoparticles.

- It is important to emphasize that the precursor used was AgNO3, which is why it should be borne in mind that the composition of growth media used for microorganisms is very important for the final results. For example, Barin Heart Infusion used for growing the mushrooms present as it contains sodium chloride may be the main factor that has led to the formation of silver chloride nanoparticles by reducing the AgNO3 precursor in the presence of *Rhodotorula Mucilaginosa, Enterobacter Faecalis, Pantoea* and *Raoultella Planticola*.

- Silver chloride nanoparticles synthesized by *Rhodotorula Mucilaginosa* have been found to decrease in size with increasing precursor concentrations. The low-cost synthesis method is reinforced by the ecological steps of the procedure. Antimicrobial activity of silver chloride nanoparticles confirms their high potential.

- Tests for antimicrobial activity of silver chloride nanoparticles have produced important results against microorganisms such as *Staphyococcus aureus, Streptococcus pyogenes, Salmonella* and *Bacillus amyloliquefaciens.*

# CHAPTER VI
# FINAL CONCLUSIONS AND OWN CONTRIBUTIONS.

## 6.1 Final conclusions

- Nanomaterial synthesis techniques, both top-down and bottom-up have revolutionized the use of nanomaterials in different areas. The potential of nanoparticles to present ecologically stable sizes and shapes increases the demand for industrial scale production.
- "Bottom-up" approaches have proven to be more favorable, which is why many nanoparticle synthesis techniques have been developed following the principle of self-assembly.
- Biosynthesis of metal nanoparticles is an interdisciplinary field ("bio-nanotechnology") that requires collaboration between physicists, chemists, biologists and engineers. The exploitation of natural resources and the implementation of these biological synthesis methods have proved to have many advantages, such as: environmental protection, simplicity of implementation in production, cost-effectiveness.
- The rate of biosynthesis production and particle monodispersity are continuously improved. The transition from bacteria to fungi as a means of developing natural "nanofabrics" is an added advantage in the synthesis of nanoparticles using microorganisms.
- The biochemical pathways involved in the synthesis of nanoparticles need to be carefully studied and the specific genes and enzymes involved must be characterized. This will help us have better control over the parameters that define the properties of a nanoparticle, such as size, shape and dispersion.
- This is the first study to underline that zinc oxide nanoparticles and micro-flowers structures could be obtained by mediation by the *Enterobacter* spp. LA9 in the presence of $Zn(NO_3)_2 \times 6H_2O$ precursor.
- Transforming zinc oxide nanoparticles into microflora, changing a single parameter (incubation time), is proposed as a cost-effective production method that could widen the range of possible applications.
- *B. amyloliquefaciens* 1853 and *B. subtillis* 10833 have the ability to synthesize extracellular silver nanoparticles by bioreduction from the 1 mM $AgNO_3$ precursor solution.
- Ag NPs synthesized using *B. amyloliquefaciens* 1853 and *B. subtilis* 10833 exhibited significant antibacterial and antifungal activity, either alone or in combination with antibiotics.
- The proposed synthesis approach presents promising potential in the optical field (refraction index of materials / colors by incorporating nanoparticles), in biochemical applications (biosensor tests, biological labels) as well as in the manufacture of conductive inks and electrothermal paints.

- Silver chloride nanoparticles were synthesized by bio-reduction. It has been shown that the aqueous enzymatic extracts of Rhodotorula Mucilaginosa, Enterobacter Faecalis, Pantoea and Raoultella Planticola, are capable of synthesizing silver chloride nanoparticles.
- It was found that silver biotransformed nanoparticles of Rhodotorula Mucilaginosa diminish in size with increasing precursor concentrations. The low-cost synthesis method is reinforced by the ecological steps of the procedure. Antimicrobial activity of silver chloride nanoparticles.
- The advantage of the proposed synthesis method lies in its economic efficiency (the broad availability of *Enterobacter, B. Amyloliquefaciens and B. Subtilis* strains and the one-step synthesis approach of reducing zinc and silver ions by enzymatic extraction without bacteria).
- The potential disadvantages of the method may include the high duration of synthesis (48 hours), reproducibility problems (due to different behaviors in enzymatic activities of bacterial strains, the presence of impurities that can inhibit bacterial activity, careful maintenance of identical synthesis conditions).

## 6.2 Own contributions

- The completion of the PhD thesis consisted of research internships at partner universities in Cyprus and Israel respectively. In order to ensure an ascending line, unparalleled in the development of this theme, I have brought value to it by optimizing some process parameters, depending on the availability at that time in the place where we are conducting the research. Thus, the results obtained by changing the parameters such as incubation time, temperature, pH, type of microorganism, precursor concentration are only the essential elements that have impressed the original contributions.
- The use of microorganisms such as Enterobacter for the synthesis of ZnO nanoparticles, Rhodotorulla Muciluginasa, Enterobacter Faecalis, Pantoea and Raoultela Planticola for the synthesis of AgCl nanoparticles represent original contributions to the green nanoparticle synthesis using microorganisms.
- Developing synergism on the antimicrobial activity of silver nanoparticles against Fluconazole resistant Candida albicans strains.
- Valorisation and optimization of the results obtained by different methods of characterization of nanoparticles.

## 6.3 Dissemination of the results

The research trajectory during the doctoral studies was realized by publishing the results in specialized journals, as well as by presenting papers at national and international conferences. The scientific publication on the dissemination of research results provides information on the heterogeneous orientation developed during the research program.

Papers published in specialty journals – ISI

- **Ghiuță, I**. Cristea, D., Croitoru, C., Kost, J., Wenkert, R., Vyrides, I. Anayiotos, A., Munteanu, D. Characterization and antimicrobial activity of silver nanoparticles, biosynthesized using Bacillus species, Applied Surface Science (2017), http://dx.doi.org/10.1016/j.apsusc.2017.09.163.
- Jinga, V., Mateescu, A.O., Cristea, D, Mateescu, G., Burducea, I., Ionescu, C., Crăciun, **I., Ghiuță**, I., Samoliă, C., Ursuțiu, D, Munteanu, D., Compositional, morphological and mechanical investigations of monolayer type coatings obtained by standard and reactive magnetron sputtering from Ti, $TiB_2$ and WC, **Applied Surface Science**, Volume 358, Part B, 15 December 2015, Pages 579–585.

Articles published in specialty journals – BDI / B+

- **Ghiuță, I.,** Cristea, D., Miloșan, I., Munteanu, D.: Synthesis of Metallic Nanoparticles Mediated by Microbes, RECENT, vol. 53pp. 177-183, 2017.
- **Ghiuță, I.,** Cristea, D., Munteanu, D.: Synthesis methods of metallic nanoparticles - an overview, Bulletin of the Transilvania University of Brasov, vol. x, 2017, Series I – Engineering Sciences, ISSN 2065-2119, pp. x
- **Ghiuta, I**., Cristea, D., Tint, D., Munteanu, D.: Surface Modification of Metallic Biomaterials Used as Medical Implants and Prostheses, Bulletin of the Transilvania University of Brasov, Vol. 8 (57) No.2- 2015, Series I – Engineering Sciences, ISSN 2065-2119, pp. 159-164
- Cristea, D., **Ghiuță, I**., Munteanu, D.: Tantalum Based Materials for Implants and Prostheses Applications, Bulletin of the Transilvania University of Brasov, Vol. 8 (57) No.2- 2015, Series I – Engineering Sciences, ISSN 2065-2119, pp. 151-158;

Articles in extenso published in Proceedings

- Pătru, M., Cristea, D., **Ghiuță, I.,** Munteanu, D.: The effect of Ti /AlN interlayers on the mechanical and tribological behavior of DLC coatings, European Conference on Heat Treatment 2015 22nd IFHTSE Congress, 20 – 22 Mai Venetia, Italia.

International Conferences attendings

- **Ghiuță I.,** Cristea D., Munteanu D., Biosynthesis of metallic nanoparticles using microorganisms,10th International Conference on Materials Science & Engineering, Brașov, Romania
- **Ghiuță**, I., D. Cristea, I. Vyrides, A. Anayiotos, D. Munteanu: Biosynthesis of zinc oxide nanoparticles using Enterobacter, 10th International Conference on Materials Science & Engineering, Brașov, Romania
- **Ghiuță, I.** ,Kost, J., Wenkert, R., Munteanu, D.: Green synthesis of silver chloride nanoparticles using Rhodotorula Mucilaginosa, 5th International Conference on Powder Metallurgy & Advanced Materials, Cluj, România
- **Ghiuță, I.** Cristea, D., Wenkert, R., Munteanu, D:: Precursor concentration influence on the morphology and antimicrobial activity of silver chloride nanoparticles. In: Book of Abstracts, 5th International Conference on Powder Metallurgy & Advanced Materials, Cluj, România,2017.
- **Ghiuță I,** Cristea D, Țînț D, Munteanu D., Surface modification of metallic biomaterials used as medical implants and prostheses, New Trends on Sensing - Monitoring - Telediagnosis for Life Sciences, Brașov, România, NT-SMT-LS September 3-5, 2015
- Cristea D., **Ghiuță I.,** Munteanu D., Thin film coatings for implants and prostheses applications, New Trends on Sensing - Monitoring - Telediagnosis for Life Sciences, Brașov, România, NT-SMT-LS September 3-5, 2015
- D. Cristea, L. Cunha**, I. Ghiuță,** N.P. Barradas, E. Alves, D. Munteanu: Photocatalytic Behviour of Magnetron Sputtered Tantalum Oxynistride Thin Films, International Conference on Materials Science and Engineering - BRAMAT 2015, 05-07 Martie 2015, Brașov, România.
- Patru, M., D. Cristea, **I. Ghiuță**, D. Munteanu , The Effect of AlN/Ti Interlayers on the Mechanical and Tribological Behaviour of DLC Coatings; European Conference of Heat Treatment 2015 & 22nd IFHSTE Congress, 20-22 May 2015, Venice, Italy

National Conferences attendings

- **Ghiuță I**, Stenturi metalice folosite în cardiologia intervențională, Conferinta Națională Creativitate și Inventică, 6 iunie 2015

## 6.4 Future research directions

- Taking into account the situations encountered during the studies, various research directions have emerged. Many of them materialized and were presented in the paper, but some define the future research directions of the subject:

- Testing the behavior of zinc oxide nanoparticles obtained by green methods by adding them to the recipes of sunscreen creams. It is also desirable to develop in the same direction titanium dioxide nanoparticles.

- Expanding the research on combining the synthesis method using microorganisms with other methods, such as: sonofragmentation, laser ablation, etc.
- Optimization of process parameters of green synthesis using microorganisms to obtain solutions with monodisperse nanoparticles, excluding the use of chemical stabilizers.
- Identification of enzymes secreted by microorganisms, which are responsible for the bioreduction of metal ions. It aims to develop and implement them in biosynthesis processes of nanoparticles.
- Deepen research and make a comprehensive comparison of the use of different nanoparticle synthesis methods and testing the unique properties they hold. Identifying the optimal method in terms of cost, time, performance. Also, account must be taken of the eco percentage in the development of the method.
- Development of green synthesis of metal nanoparticles using different plant extracts. It is also desirable to achieve a parallel between the animicrobial activity developed by nanoparticles obtained by microorganism-mediated green synthesis as well as green synthesis mediated by plant extracts.

## SELECTIVE REFERENCES

[1] Abhijeet, M. Meryam, S.: Alpha-Amylase Mediated Synthesis of Silver Nanoparticles, Science of Advanced Materials, vol. 4, iss. 1, pp. 143-146, 2012.

[4] Ahmad, A., et al.: Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, Rhodococcus species. In: Nanotechnology,vol. 14, no. 7, 2003.

[5] Ahmed, S., et al.: A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. In: Journal of Advanced Research, ISSN: 2090-1232, vol. 7, is. 1, pp. 17–28, 2016.

[11] Armarego, W. L. F.: Nanomaterials and Nanotechnology, Purification of Laboratory Chemicals, Butterworth-Heinemann pp.1065-1106, 2017.

[14] Asokan, A. et al., Preparation and Characterization of Zinc Oxide Nanoparticles and a Study of the Anti-microbial Property of Cotton Fabric Treated with the Particles, JTATM 6 (2010) 723-729.

[17] Bai, H.-J. Z.-M. Zhang, J. Gong, Biological synthesis of semiconductor zinc sulfide nanoparticles by immobilized Rhodobacter sphaeroides, Biotechnol Lett. 28, 1135–1139, 2006.

[19] Balasooriya, E.R., et al.: Review Article Honey Mediated Green Synthesis of Nanoparticles: New Era of Safe Nanotechnology. In: Journal of Nanomaterials, vol. 2017, Article ID 5919836, 10 pages, 2017.

[26] Berta Domènech, et al.: Bifunctional Polymer-Metal Nanocomposite Ion Exchange Materials. In: Ion Exchange Technologies, Editors: Ayben Kilislioğlu, pp.35-72, 2012.

[28] Bhushan, B.: Introduction to Nanotechnology. In: Bhushan B. (eds) Springer Handbook of Nanotechnology. ISSN: 2522-8692 Springer, Berlin, Heidelberg, pp. 1-12, 2010.

[37] Carvalho, J.F., et al.: Synthesis of magnetite nanoparticles by high energy ball milling. In: Applied Surface Science, ISSN: 0169-4332, vol. 275, pp. 84– 87, 2013.

[38] Castro, L., et al.: Mechanism and Applications of Metal Nanoparticles Prepared by Bio-Mediated Process. In: Reviews in Advanced Sciences and Engineering (RASE), ISSN: 2157-913X, vol. 3, pp. 1–18, 2014.

[40] Chauhan, R. A. Reddy, J. Abraham, Biosynthesis and antimicrobial potential of silver and zinc oxide nanoparticles using Candida diversa strain JA1, Der Pharma Chemica, vol. 6, iss. 3, pp. 39-47, 2014.

[46] Chokriwal, A. et al.: Biological Synthesis of Nanoparticles Using Bacteria and Their Applications. In: American Journal of Pharmatech Research (Am. J. PharmTech Res), ISSN: 2249-3387, vol. 4, iss. 6, pp. 38-61, 2014.

[48] Cunningham, A. and T. Bürgi: Bottom-up Organisation of Metallic Nanoparticles. In: C. Rockstuhl, T. Scharf (eds.), Amorphous Nanophotonics, Nano-Optics and Nanophotonics, pp. 1-37, 2013.

[51] Dar, M.A., Ingle, A., Rai, M.,Enhanced antimicrobial activity of silver nanoparticles synthesized by Cryphonectria sp. evaluated singly and in combination with antibiotics. In: Nanomedicine, ISSN: 1549-9642, vol. 9, iss. 1, pp. 105-114, 2013.

[61] Edson Roberto Leite, Nanocrystals Assembled from the Bottom Up. In: Encyclopedia of Nanoscience and Nanotechnology Edited by H. S. Nalwa, vol 6, pp. 537–554, 2004.

[65] Fayaz, A.M., et al.: Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. In: Nanomedicine: Nanotechnology, Biology and Medicine, ISSN: 1549-9642, vol. 6, iss. 1, pp. 103-109, 2010.

[67] Feynman, Richard P.: There's Plenty of Room at the Bottom. In: Caltech Engineering and Science, ISSN 0013-7812, vol. 23, no. 5, pp. 22-36, 1960.

[73] Gericke, M., Pinches, A.: Biological synthesis of metal nanoparticles. In: Hydrometallurgy, ISSN: 0304-386X, vol. 83, iss. 1-4, pp. 132–140, 2006.

[74] **Ghiuță, I.** Cristea, D. Munteanu, D. Biosynthesis of metallic nanoparticles using microorganisms. In: Book of abstract,10th International Conference on Materials Science & Engineering, Brașov, Romania, 2017.

[75] **Ghiuță, I.** Cristea, D., Croitoru, C., Kost, J., Wenkert, R., Vyrides, I. Anayiotos, A., Munteanu, D. Characterization and antimicrobial activity of silver nanoparticles, biosynthesized using Bacillus species, Applied Surface Science 2017,

[76] **Ghiuță, I.** Cristea, D., Kost, J., Wenkert, R., Munteanu, D.: Green synthesis of silver chloride nanoparticles using Rhodotorula Mucilaginosa, In: Book of abstract, 5th International Conference on Powder Metallurgy & Advanced Materials, Cluj, România, 2017

[77] **Ghiuță, I.** Cristea, D., Wenkert, R., Munteanu, D:: Precursor concentration influence on the morphology and antimicrobial activity of silver chloride nanoparticles. In: Book of Abstracts, 5th International Conference on Powder Metallurgy & Advanced Materials, Cluj, România,2017.

[78] **Ghiuță, I.,** Cristea, D. Vyrides, I. Anayiotos, A. Munteanu D.: Biosynthesis of zinc oxide nanoparticles using Enterobacter. In: Book of abstract, 10th International Conference on Materials Science & Engineering, Brașov, Romania,2017

[79] **Ghiuță, I.,** Cristea, D., Munteanu, D.: Synthesis methods of metallic nanoparticles - an overview, Bulletin of the Transilvania University of Brasov, Series I – Engineering Sciences, ISSN 2065-2119, vol. 10, iss. 59, no. 2, pp. 133, 140, 2017.

[80] **Ghiuță, I.,** Cristea, D., Miloșan, I., Munteanu, D.: Synthesis of Metallic Nanoparticles Mediated by Microbes, RECENT, vol. 53pp. 177-183, 2017.

[81] Ghorbani, H.R. et al.: A Review of Methods for Synthesis of Al Nanoparticles. In: Oriental Journal of Chemistry (OJC), ISSN: 2231-5039, vol. 30, is. 4, pp. 1941-1949, 2014.

[92] Habiba K, Makarov VI, Weiner BR, Morell G.: Fabrication of Nanomaterials by Pulsed Laser Synthesis. Manufacturing Nanostructures, Publisher: One Central Press (OCP), Editors: Waqar Ahmed, Nasar Ali, pp. 263-291, 2014.

[95] Haiss, W. Thanh N.T.K., Aveyard, J. Fernig, D.G. Determination of size and concentration of gold nanoparticles from UV-Vis spectra, Anal. Chem. vol. 79, pp. 4215–4221, 2007.

[100] Heiligtag, F.J., Niederberger, M.: The fascinating world of nanoparticle research. In: Materials Today, vol. 16, iss. 7-8, pp. 262-271, 2013.

[103] Hu, W. et al., In situ synthesis of silver chloride nanoparticles into bacterial cellulose membranes, Mater. Sci. Eng. C. vol. 29, pp. 1216–1219, 2009.

[104] Husseiny, M.I., et al.: Biosynthesis of gold nanoparticles using Pseudomonas aeruginosa. In: Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy (SAA), ISSN 1386-1425, vol. 67, p. 1003–1006, 2007.

[106] Iacoban, ]. C. Macoveanu, M. A Comparison of Argentometric Titration and Spectrophotometric Determination of Chloride Concentration in Precipitation Samples, Roum. Biotechnol. Lett. vol. 10, pp. 2105–2109, 2005.

[111] Javed Musarra, A. Production of antimicrobial silver nanoparticles in water extracts of the fungus Amylomyces rouxii strain KSU-09, Bioresour. Technol. vol. 101, pp. 8772–8776, 2010.

[116] Jha, A.K., Prasad, K., Kulkarni, A.R.: Synthesis of TiO2 nanoparticles using microorganisms. Colloids and Surfaces B: Biointerfaces . ISSN 0927-7765, vol.71 p. 226–229, 2009.

[144] Mishra, V., et al.: A Review on Green Synthesis of Nanoparticles and Evaluation of Antimicrobial Activity. International Journal of Green and Herbal Chemistry (IJGHC), ISSN: 2278-3229, vol.3, no.1, pp. 081-094, 2013.

[146] Mittal, A.K., Chisti, Y., Banerjee, U.C.: Synthesis of metallic nanoparticles using plant extracts, Biotechnol. Adv., ISSN: 0734-9750, vol. 31, is. 2, pp. 346-56, 2013.

[147] Mody, V.V.: Introduction to metallic nanoparticles. In: Journal of Pharmacy and Bioallied Science (J. Pharm. Bioallied Sci.), ISSN: 0975-7406, vol. 2, is. 4, pp. 282–289, 2010.

[152] Pantidos, N.,Horsfall, L.E.: Biological Synthesis of Metallic Nanoparticles by Bacteria, Fungi and Plants. In: Journal of Nanomedicine and Nanotechnology (J Nanomed Nanotechnol), ISSN: 2157-7439, vol. 5, iss. 5, pp. 233-242, 2014.

[164] Rai M.; Yadav; A. Gade, A. Silver nanoparticles as a new generation of antimicrobials, Biotechnology Advances vol. 27, pp. 76-83, 2009.

[167] Ramani, M. S. Ponnusamy, C. Muthamizhchelvan, From zinc oxide nanoparticles to microflowers: A study of growth kinetics and biocidal activity, Mater. Sci. Eng., C vol. 32, pp. 2381–2389, 2012.

[178] Saifuddin, N. Wong, C. W. Nur Yasumira A. A.: Rapid Biosynthesis of Silver Nanoparticles Using Culture Supernatant of Bacteria with Microwave Irradiation, E-J. Chem. vol. 6, iss. 1, pp. 61-70, 2009.

[179] Salata, O.V.: Applications of nanoparticles in biology and medicine, Journal of Nanobiotechnology, ISSN: 1477-3155 , vol. iss.3, 6 pages, 2004.

[182] Sanghi, R., Verma, P.: A facile green extracellular biosynthesis of CdS nanoparticles by immobilized fungus. In: Chemical Engineering Journal, ISSN 1385-8947, vol. 155, p. 886–891, 2009.

[189] Shamsuzzaman, et al.: Biological synthesis of ZnO nanoparticles using C. albicans and studying their catalytic performance in the synthesis of steroidal pyrazolines. In: Arabian Journal of Chemistry, ISSN 1878-5352, vol. 10, suppl. 2, p. 1530–1536, 2017.

[193] Singh, G., et al.: Synthesis of ZnO nanoparticles using the cell extract of the cyanobacterium, Anabaena strain L31 and its conjugation with UV-B absorbing compound shinorine. In: Journal of Photochemistry and Photobiology B: Biology, ISSN 1011-1344, vol. 138 p. 55–62, 2014.

[195] Singh, P., Zhang, D., Yang, D.-C.: Biological Synthesis of Nanoparticles from Plants and Microorganisms. In: Trends in Biotechnology, ISSN: 0167-7799, vol. 34, iss. 7, pp. 588-599, 2016.

[199] Soriano, L. M., Zougagh, M., Valcárcel, M., Ríos, Á.: Analytical Nanoscience and Nanotechnology: Where we are and where we are heading. In: Talanta, ISSN: 1873-3573 vol. 177, pp. 104-121, 2018.

[208] Taniguchi, N.: On the Basic Concept of 'Nano-Technology. In: Proceedings of the International Conference on Production Engineering (Proc. Intl. Conf. Prod. Eng.), Tokyo, Part II, Japan Society of Precision Engineering, 1974.

[210] Teimuri-Mofrad, R., et al: Green synthesis of gold nanoparticles using plant extract: Mini-review. In: Nanochemistry Research (Nanochem. Res.), ISSN: 2423-818X, vol. 2, iss. 1, Winter and Spring, pp. 8-19, 2017.

[215] Tsibakhashvili, N., et al.: Synthesis of Silver Nanoparticles Using Bacteria. In: Nano Studies, ISSN 1987-8826, vol. 2, p. 179-182, 2010.

[216] Tsuzuki, T., Commercial scale production of inorganic nanoparticles. In: International Journal of Nanotechnology (IJNT), ISSN online: 1741-8151, vol. 6, iss. 5-6, pp. 567-578, 2009.

[220] Vadlapudi, V., et al.: Synthesis of Green Metallic Nanoparticles (NPs) and Applications. In: Oriental Journal of Chemistry, ISSN: 2231-5039, vol. 29, no. 4, pp. 1589-1595, 2013.

[222] Wahab, R. Photocatalytic activity of zinc oxide micro-flowers synthesized via solution method, Chem. Eng. J. vol. 168, pp. 359–366, 2011.

[230] Wiggins, P. A. et al.: High flexibility of DNA on short length scales probed by atomic force microscopy. In: Nature Nanotechnology (Nat. Nanotechnol.), ISSN: 1748-3395, vol 1, pp. 137–141, 2006.

[249] ***https://www.nobelprize.org/educational/physics/microscopes/tem/ accesat la 17.04.2017

[253] ***http://www.indiastudychannel.com/resources/146681-Principle-working-and-applications-of-UV-spectroscopy.aspx, accesat la 08.01.2017

## Abstract (RO)

Scopul tezei de doctorat îl reprezintă studiul nanoparticulelor metalice, reliefat de sinteza, caracterizarea și aplicabilitatea acestora, realizate în vederea dezvoltării și îmbunătățirii activității antimicrobiene. Pentru sinteza de nanoparticule au fost utilizate diferite microorganisme capabile să înlocuiască reducătorii și stabilizatorii chimici. Astfel, s-a demonstrat că bacterii ca *Enterobacter* și *Bacillus* sunt capabile sa medieze sinteza de nanoparticule de oxid de zinc, respectiv de argint. De asemenea, microorganisme precum *Rhodotorulla, Raoultella, Pantoea* și *Enterococcus* au fost folosite pentru sinteza nanoparticulelor de clorură de argint. Rezultatele obținute au fost optimizate prin adaptarea unor parametrii de proces, ca timpul de incubare, temperatură sau concentrația molară a precursorului. Nanoparticulele au fost caracterizate atât din punct de vedere morfologic, cât și structural. Pe parcursul studiilor a fost testată activitatea antimicrobiană a nanoparticulelor biosintetizate și a fost demonstrată capacitatea acestora de a dezvolta sinergismul în prezența unor antibiotice.

## Abstract (ENG)

The aim of the PhD thesis is the study of metal nanoparticles, highlighted by the synthesis, characterization and applicability of these particles, with the purpose of developing and improving their antimicrobial activity. For the synthesis of nanoparticles, various microorganisms capable of replacing chemical reducers and stabilizers have been used. Thus, it has been shown that bacteria such as *Enterobacter* and *Bacillus* are capable of mediating the synthesis of zinc oxide and silver nanoparticles. Moreover, microorganisms such as, *Rhodotorulla, Raoultella, Pantoea* and *Enterococcus* have been used for the synthesis of silver chloride nanoparticles. The results obtained were optimized by adapting some process parameters, such as incubation time, temperature, or molar concentration of the precursor. The nanoparticles have been characterized both morphologically and structurally. During the studies, the antimicrobial activity of biosynthesized nanoparticles was tested and their ability to develop synergism in the presence of antibiotics was demonstrated.


# Ghiuță Ioana

Strada Sporturilor, Bloc 2A, Ap 26, Buzău, Romania,
Naționalitate: Română; Data nașterii: 06.01.1990
ioana.ghiuta@unitbv.ro ghiuta.ioana@yahoo.com
Telefon: +40 734 219 708


## EXPERIENȚĂ PROFESIONALĂ

Universitatea Transilvania din Brașov, Facultatea de Știința și Ingineria Materialelor, *Cadru asociat, 2017 – prezent*

## EDUCAȚIE ȘI FORMARE PERSONALĂ

| | |
|---|---|
| Perioada | Octombrie 2014 – Ianuarie 2018 |
| Numele instituției de învățământ | Universitatea Transilvania din Brașov, Facultatea de Știința și Ingineria Materialelor |
| Poziția ocupată | Doctorand |
| Titlul tezei | Cercetări privind biosinteza nanoparticulelor metalice utilizând microorganisme |
| Subiecte aprofundate | Sinteza nanoparticulelor metalice, Caracterizarea nanoparticulelor metalice. |
| Perioada | Ianuarie 2017 – Iunie 2017 |
| Numele instituției de învățământ | Ben-Gurion University from Negev, Beer-Sheva, Israel |
| Poziția ocupată | Student-Doctorand (Erasmus) |
| Perioada | Octombrie 2015 – Decembrie 2015 |
| Numele instituției de învățământ | Cyprus University of Technology, Limasol, Cipru |
| Poziția ocupată | Student-Doctorand (Erasmus) |
| Perioada | 2012-2014 |
| Numele instituției de învățământ | Universitatea Transilvania din Brașov, Facultatea de Știința și Ingineria Materialelor |
| Specializarea | Ingineria și Managementul Materialelor Avansate Metalice, Ceramice si Compozite |
| Diploma obținută | Diplomă de master în Domeniul Ingineriei Materialelor |
| Subiecte aprofundate | Metode de caracterizare a materialelor, Managementul calității |
| Perioada | 2008-2012 |
| Numele instituției de învățământ | Universitatea Transilvania din Brașov, Facultatea de Știința și Ingineria Materialelor |
| Specializarea | Inginerie Economică în Domeniul Mecanic |
| Diploma obținută | Diplomă de Inginer în Domeniul Inginerie și Management |
| Subiecte aprofundate | Știința Materialelor, Nanomateriale, Tratamente Termice, Proiectare CAD, Economie |
| Perioada | 2008-2011 |
| Numele instituției de învățământ | Universitatea Spiru Haret, Facultatea de Științe Juridice și Administrative, Brașov |
| Specializarea | Administrație Publică |
| Diploma obținută | Licențiat în Științe Administrative |
| Subiecte aprofundate | Drept civil, drept penal, Economie |
| Perioada | 2013-2014 |
| Numele instituției de învățământ | Universitatea Transilvania din Brașov |
| Curs Postuniversitar | Poluarea, Protecția şi Managementul Mediului |
| Diploma obținută | Certificat de atestare a competențelor profesionale |
| Perioada | 2008-2011 |
| Numele instituției de învățământ | Universitatea Transilvania din Brașov |
| Curs; Diploma obținută | Modul Psihopedagogic - Certificat de absolvire |

# Ghiuță Ioana

Street Sporturilor, Bld 2A, Ap 26, Buzau, Romania,
Națională: Romanian; Birth date: 06.01.1990
ioana.ghiuta@unitbv.ro ghiuță.ioana@yahoo.com
Phone: +40 734 219 708


## PROFESSIONAL EXPERIENCE

Transylvania University of Brasov, Faculty of Materials Science and Engineering, *Assistant, 2017 – present*

## EDUCATION AND PERSONAL TRAINING

| | |
|---|---|
| Period | October 2014 – January 2018 |
| Name of the educational institution | Transilvania University of Brasov, Faculty of Materials Science and Engineering |
| Role | PhD |
| Thesis title | Research on the biosynthesis of metallic nanoparticles using microorganisms |
| In-depth topics | Synthesis of metallic nanoparticles, Characterization of metallic nanoparticles |
| Period | January 2017 – June 2017 |
| Name of the educational institution | Ben-Gurion University from Negev, Beer-Sheva, Israel |
| Role | PhD-Student - (Erasmus) |
| Period | October 2015 – December 2015 |
| Name of the educational institution | Cyprus University of Technology, Limassol, Cyprus |
| Role | PhD-Student-(Erasmus) |
| Period | 2012-2014 |
| Name of the educational institution | Transilvania University of Brasov, Faculty of Materials Science and Engineering |
| Specialization | Engineering and management of advanced, metallic, ceramic and composite materials. |
| Title of qualification awarded | |
| In-depth topics | Master degree in Materials Engineering, Characterization methods of materials. Quality Management |
| Period | 2008-2012 |
| Name of the educational institution | Transilvania University of Brasov, Faculty of Materials Science and Engineering |
| Specialization | Economical Engineering in Mechanical Field |
| Title of qualification awarded | Engineer in the field of Engineering and Management |
| In-depth topics | Materials Science, Nanomaterials, Thermic treatments, CAD Projection, Economics |
| Period | 2008-2011 |
| Name of the educational institution | Spiru Haret University, Faculty of Juridic and Administrative Sciences, Brasov |
| Specialization | Public Administration |
| Title of qualification awarded | Licensed in Administrative Sciences |
| In-depth topics | Civil rights, Criminal Law, Economics |
| Period | 2013-2014 |
| Name of the educational institution | Transilvania University of Brasov |
| Postgraduate course | Pollution, Protection and Management of the Environment |
| Title of qualification awarded | Certificate for professional competences |
| Period | 2008-2011 |
| Name of the educational institution | Transilvania University of Brasov, |
| Course; Obtained diploma | Psychopedagogical module - Certificate of graduating |