MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES
IN PURE AND APPLIED SCIENCES

BIOTRANSFORMATION STUDIES WITH *CURVULARIA LUNATA*

DENİZ YILMAZ

MASTER THESIS

Department of Bioengineering

Thesis Supervisor
Prof. Dr. Berna Sarıyar AKBULUT

Thesis CO- Supervisor
Assoc. Prof. Dr. Nihat Alpagu SAYAR

ISTANBUL, 2019

MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES
IN PURE AND APPLIED SCIENCES

BIOTRANSFORMATION STUDIES WITH *CURVULARIA LUNATA*

DENİZ YILMAZ

524215022

MASTER THESIS

Department of Bioengineering

Thesis Supervisor
Prof. Dr. Berna Sarıyar AKBULUT

Thesis CO- Supervisor
Assoc. Prof. Dr. Nihat Alpagu SAYAR

ISTANBUL, 2019

MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES IN PURE AND APPLIED SCIENCES

Deniz YILMAZ, a Master of Science student of Marmara University Institute for Graduate Studies in Pure and Applied Sciences, defended her thesis entitled **"Biotransformation of Different Alkaloids and Antibiotics into Biologically Active Natural Products on** .... and has been found to be satisfactory by the jury members.

Jury Members

Prof. Dr. Berna SARIYAR AKBULUT (Advisor)
Marmara University ....(SIGN) .... B. Sarıyar

Assist. Prof. Hilal TAYMAZ NİKEREL (Jury Member)
İstanbul Bilgi Üniversitesi ....(SIGN) ....

Prof. Dr. Dilek KAZAN (Jury Member)
Marmara Üniversitesi ....(SIGN) ....

APPROVAL

Marmara University Institute for Graduate Studies in Pure and Applied Sciences Executive Committee approves that Deniz YILMAZ be granted the degree of Master of Science in Department of Bioengineering, Bioengineering Program .... (Resolution no:

Director of the Institute
Prof. Dr. Bülent EKİCİ

ACKNOWLEDGMENTS

It was a great pleasure to take part in the team of Prof. Berna Sarıyar Akbulut. I would like to thank every team member who contributed to the success of this thesis. Most importantly, I would like to thank my mentor, Prof. Berna Sarıyar Akbulut, for her unflagging support and guidance throughout my M.Sc. studies at Marmara University. Without her allegiance, this thesis would not be achievable. Her attitude towards research and her students has always inspired me and she has set a perfect example for me to follow in my future career.

I would also like to extend my gratitude to Prof. Alp Sayar and Assoc. Prof. Alpagu Sayar, for their helpful guidance. In addition, I am thankful to my laboratory group members for their collaboration and contribution to this thesis.

Finally, my graduation would not be possible without all blessing and support from my family, who help me in everything and always provide me with their greatest love and willpower until the completion of my studies.

This work is supported by Marmara University, Scientific Research Projects Committee (FEN-C- 070317-0110).

CONTENTS

BIOTRANSFORMATION STUDIES WITH *CURVULARIA LUNATA*

ÖZET

Yeni sınıf antimikrobiyal ilaç tasarımında; biyolojik olarak aktif bitki ekstratları, özütlenmiş bitki bileşikleri ve bitki türevleri önemli rol oynamaktadırlar. Çoklu ilaca dirençli mikro-oranizmaların sayısındaki artış, yeni antimikrobiyal ajanlara ve hali hazırda kullanılan antibiyotiklerin geliştirilmesine olan ihtiyacı ortaya çıkarmaktadır. Bu bağlamda, suda çözünebilen ve çeşitli bakteri ve küfe karşı biyolojik aktiviteye sahip olan berberin, -bir bitki özütü- yeni bir antimikrobiyal ürünün geliştirilmesinde ümit vadeden bir alkaloittir. Diğer taraftan, dünya genelinde artan antimikrobiyal dirence karşı yeni sınıf antibiyotiklerin geliştirilmesinin yanı sıra, polimiksin antibiyotikleri de yeniden önem kazanmaktadırlar. Günümüzde polimiksin antibiyotikleri yüksek nörotoksisite ve nefrotoksisiteye sahip olmalarından ötürü son çare olarak kullanılmaktadırlar. Bu tezin amacı, farmakolojik etkilerinden dolayı son günlerde önemli hale gelmiş berberin ve polimiksin-B üzerinde *Curvularia lunata* ile mikrobiyal transformasyon çalışmaları gerçekleştirerek, daha düşük nörotoksisite ve nefrotoksisiteye sahip polar polimiksin-B türevi antimikrobiyaller ve daha yüksek antimikrobiyal etkiye sahip berberin türevleri antimikrobiyaller elde etmektir. Tez iki ana adımdan oluşmaktadır: (1) berberin türevlerinin antimikrobiyal etkilerini arttırmak için yapılacak biyotransformasyon çalışmaları (2) polimiksin-B antibiyotiğinin toksisitesini düşürmek ve antimikrobiyal etkisini arttırmak için yapılan biyotransformasyon çalışmaları. Yaptığımız çalışmalar sonucunca, patates dekstroz besi yerinde büyütülen *C. lunata*'nın polimiksin-B'de yaptığı değişiklik, büyük olasılıkla karmaşık yapısından dolayı, belirlenememiştir. Diğer taraftan bu organizmanın yine patates dekstroz besi yerinde 14 gün inkübasyonu sonucunda, 0.35 mg/ml berberini %94 oranında ve 1 mg/ml berberini %15 oranında parçaladığı gözlemlenmiştir. Bu esnada berberin olan ortamda *C. lunata*'nın daha fazla lakkaz ürettiği belirlenmiştir.

Anahtar Kelimeler: antimikrobiyal direnç, mikrobiyal transformasyon, enzimatik transformasyon, antibiyotik geliştirme, ilaç dizaynı

BIOTRANSFORMATION STUDIES WITH *CURVULARIA LUNATA*

ABSTRACT

The use of biologically active plant extracts, isolated plant compounds and plant derivatives are essential in antimicrobial drug designing. Emerge of multidrug resistant microorganisms results in the continuous and urgent need to improve current antibiotics and to develop new antimicrobial agents. In this sense, berberine, which exhibits antimicrobial activity against several types of bacteria and fungi, is a valuable source in the search of novel and effective antimicrobial compounds. On the other hand, with the worldwide increased pace in resistance development, polymyxin antibiotics have regained importance. Today, polymyxin antibiotics are commonly used as a last resort because due to their high neurotoxicity and nephrotoxicity. The aim of this thesis is to perform microbial transformation with *Curvularia lunata* to synthesize polar polymyxin-B derivatives with less neurotoxicity and nephro-toxicity and berberine derivatives with higher antimicrobial activity. To this end, this thesis consists of two major sections: (1) biotransformation to increase the antimicrobial effect of berberine, (2) biotransformation to reduce the toxicity of Polymyxin-B antibiotics. Our results have shown that fungal transformation of polymyxin-B could not be monitored probably due to its complex nature. However, *C. lunata* had the ability of consume berberine. 94% of 0.35 mg/ml berberine and 15% for 1 mg/ml berberine found in the growth media was degraded after 14 days of incubation. The synthesis of laccase by *C. lunata* was higher in the presence of berberine.

Keywords: antimicrobial resistance, microbial transformation, enzymatic development, antibiotic development, drug designing

List of Symbols and Abbreviations

ABTS	: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ATCC	: American Type Culture Collection
BIA	: Benzyl-Isoquinoline Alkaloids
CYP	: Cytochrome
$CHCl_3$	: Chloroform
EtOAc	: Ethyl Acetate
HPLC	: High Performance Liquid Chromatography
K_2HPO_4	: Potassium hydrogen phosphate
MeOH	: Methanol
mM	: Millimolar
NADH	: Nicotinamide adenine dinucleotide
nm	: Nanometer
$(NH_4)_2SO_4$	: Ammonium sulfate
OD	: Optical density
PDA	: Potato Dextrose Agar
PDB	: Potato Dextrose Broth
rpm	: Revolutions per minute
SAR	: Structure-Activity Relationship
QSAR	: Quantitative Structure-Activity Relationship
TLC	: Thin Layer Chromatography
UV	: Ultraviolet
w/v	: Weight per volume
v/v	: Volume per volume

List of Figures

List of Tables

CHAPTER 1

1. INTRODUCTION

The effective prevention and treatment of an ever-expanding scope of infections, caused by bacteria, parasites, viruses and fungi, is threatened by antimicrobial resistance. This is one of the most serious threats to global public health, which demands action from all governmental sectors, as well as from wider society [1]. Antimicrobial resistance is present in all parts of the world, and some pathogens have even become resistant to multiple types of antibiotics, as well. Furthermore, new resistance mechanisms arise and spread globally. This means that infections from resistant bacteria are now very common [2]. Resistance to available drugs is progressively increasing at a high rate even though pharmaceutical industries have introduced a considerable number of novel antimicrobial drugs in recent years [1]. To help the combat against antimicrobial resistance; natural products (such as terrestrial plants, vertebrates, invertebrates, microorganisms and marine organisms) are in focus in order to find new antimicrobial drug classes. Another possible approach to overcome antimicrobial resistance is the reassessment of current antibiotics discovered in the 1950's to 1970's by modifying or transforming them into other biologically active compounds.

Natural products can be classified according to their chemical nature as alkaloids, terpenoids, polyketides and non-ribosomal peptides [3]. Alkaloids are low molecular weight and complex nitrogen containing organic compounds that are derived from a wide range of sources, including plants, microorganisms and marine organisms [4]. Some of these have already been successfully developed into drugs, that target a broad range of diseases (infections, malaria, hypertension, cancer) and disorders like Parkinson's disease [5].

The first discovery of polymyxin and its derivatives was through the isolation of the substrate polymyxa from *Bacillus* in early 1947 [3]. They were found to possess an exceptional activity against various bacteria and fungi. However, in the 1970's, their use in clinical practice dramatically decreased due to their adverse effects, mainly neurotoxicity and nephrotoxicity. Nevertheless, in recent years, polymyxin-B and polymyxin-E re-entered clinical use and have become last-resort antibiotics for multidrug resistant infections [4]. Polymyxins are classified as positively charged macrocyclic peptide antibiotics. They are composed of a heptapeptide ring and three amino acid exocyclic tails capped by a mixture of two fatty acids. The membrane-anchoring component of the lipopolysaccharide, which is found in the outer membrane of bacteria, initiates the antimicrobial activity of the polymyxin antibiotics. The displacement of membrane-stabilizing divalent cations, along with the insertion of the fatty acyl chain, both expands and weakens the bacterial outer membrane which causes cell leakage and allows polymyxin entry [5]. After polymyxin entry, NADH-quinone oxidoreductase inhibition and hydroxy radical generation take place in the cell [6]. Recently, with the alteration of the fatty acid tail and substitution / removal of the some amino-acids' exocyclic tails, novel polymyxin derivatives with lower toxicity have developed [7].

Chemical modifications catalyzed by microbial cells or enzymes isolated from microorganisms are defined as biotransformation reactions. According to the biocatalyst used, biotransformations can be divided into two groups: whole cell systems (microbial transformation) and cell free systems (in vitro biosystems). Different from whole cell systems, cell free systems contain cell extracts and purified enzymes [11]. With cell free systems, very high product yields can be accomplished without the formation of by-products by eliminating and re-designing the undesired side pathways. Furthermore, using these systems, it is possible to accomplish various reactions which cannot be accomplished using whole cell systems [12].

Biotransformations have been used for decades to produce various chemicals used in pharmaceutical, agrochemical and food industries. The application areas of biotransformations can be classified as drug metabolism, factor in multidrug resistance, cancer chemotherapy, environmental science and bioremediation [13]. In the pharmaceutical industry, the general consideration for drug biotransformation is to decrease the toxicity of a drug and to transform it into a more easily excreted and polar metabolite. Yet, this phenomenon can also be used to form metabolites which have greater pharmacological activity than the parent compound [9].

In the light of all this information, the scope of this study is to investigate biotransformation of polymyxin-B and berberine by *Curvularia lunata*. Toxicity-activity investigations of chemically synthesized polymyxin derivatives have been reported [4]. However, there has yet been no report on biotransformation of polymyxins to reduce their toxicity and improve activity. Furthermore, there has been no work on the biotransformation of berberine by *C. lunata* to get more active compounds.

1.1. Bacterial Resistance to Antimicrobial Agents

In order to combat with infections, many antibiotics have been developed since the 20th century. Antibacterial agents are able to stop bacterial growth and kill bacteria by affecting four main mechanisms in bacterial systems: interference with bacterial cell wall biosynthesis, inhibition of bacterial protein synthesis, interference with nucleic acid synthesis, and inhibition of metabolic pathways [14]. However, as a result of the overuse and misuse of current drugs, bacteria have developed a number of resistance mechanisms in order to protect themselves from antibacterial agents [15]. Antibiotic resistance has been regarded as one of the three most important public health threats of the 21st century by the World Health Organization [1]. Approximately 700,000 people die annually due to microbial infections and by 2050 this figure is projected to increase to about 10 million [16]. Figure 1 depicts the discovery of antibiotics and evolution of resistance to antibiotics.

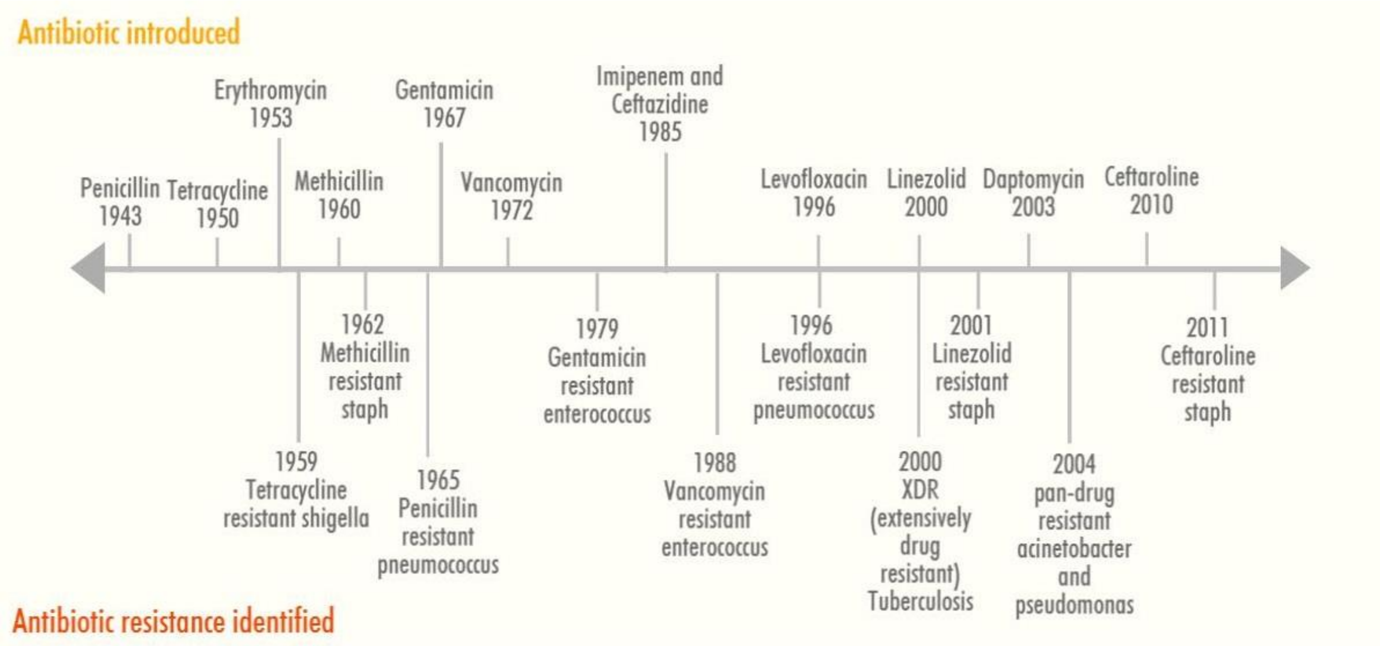


Figure 1. Discovery of antibiotics and evolution of resistance to antibiotics (CDC)

Bacteria develop resistance different mechanisms before or after the antimicrobial agent enters the cell [17]. Bacterial resistance may develop through mutations in the structural or regulatory genes (vertical evolution). Alternatively, resistance may be acquired through horizontal acquisition of foreign genetic material that codes for resistance in the same species or different species or genera (horizontal evolution). Both of these events can be associated with the emergence and serious spread of resistance [18]. Figure 2 shows how bacteria may resist the presence of antibiotics.

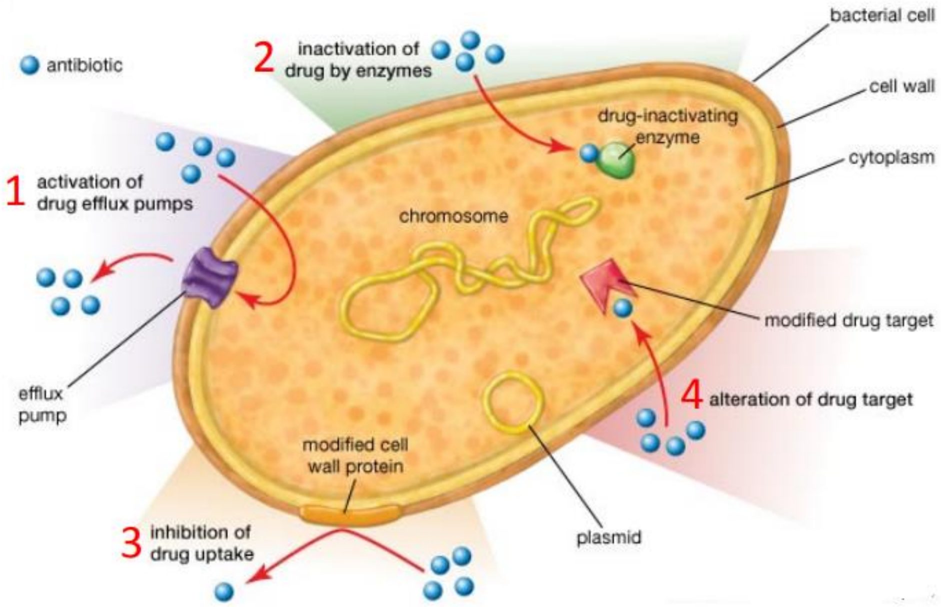


Figure 2. Schematic Representation of Bacterial Resistance [19]

Bacteria can modify the action of antibiotics through 1) modifications of the antimicrobial target (decreasing the affinity for the antimicrobial agent) 2) a decrease in the drug uptake 3) up-regulation of the outer membrane protein channels to extrude the antibiotic or 4) alterations in metabolic pathways [2].

1.2. Drug Design

An effective way to combat with antimicrobial resistance is to develop and design novel antimicrobial agents. There are two methodologies for drug design. Drug discovery can be based on targeting a specific disease or by considering the target after optimizing a molecule, which has known to have biological activity. Drug precursors have been investigated in many ways, including isolation from natural products, alteration of natural products, and modification of known synthetic chemicals or structure-activity relationship studies. Precursor compounds are generally not suitable for direct clinical trials either because they are highly toxic or they can exhibit undesirable side-effects. Once the target or the precursor molecule has been identified, a pathway is selected to obtain the final product using structure-activity relationships, quantitative structure-activity relationships, combinatorial chemistry, and computer-assisted chemistry programs based on a number of common valid rules [20]. The cycle of drug discovery can be seen in figure 3 and 4.

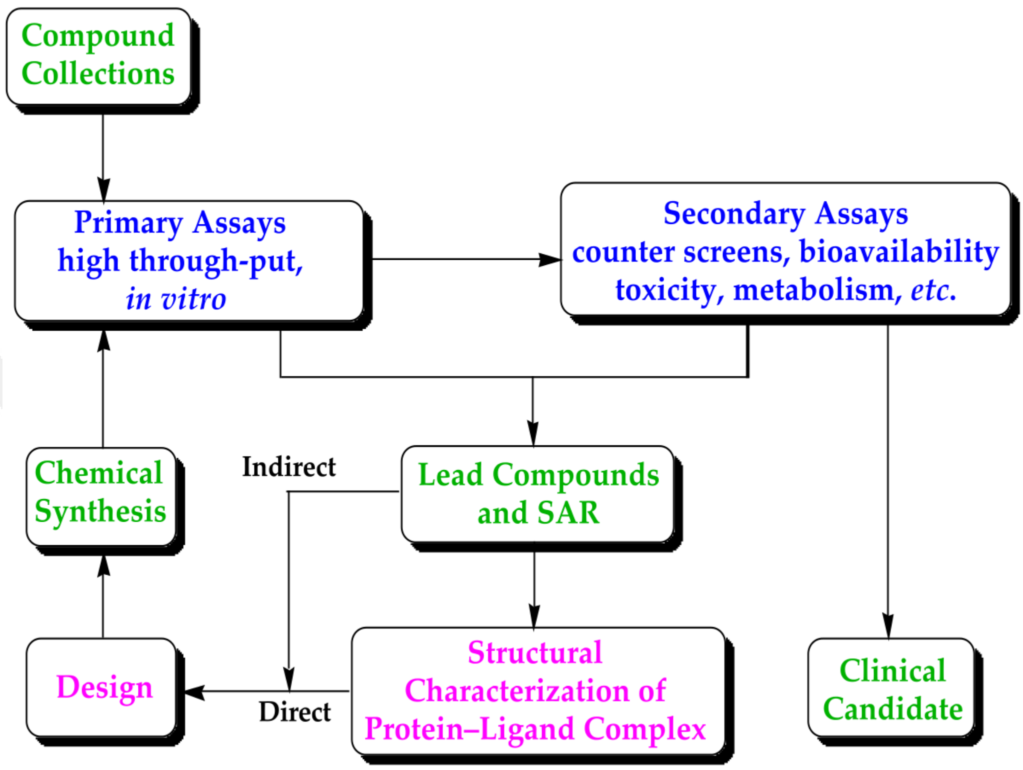


Figure 3. Drug discovery cycle [16]

Chemically synthesized molecules or natural products may constitute precursor molecules. Although precursor molecules from natural products can be used directly, usually they need modifications for improvement of their activities because; despite their high biological activities, natural products may be very toxic. Modification of the natural products may be achieved either by chemical reactions or by biotransformation reactions. Biotransformation reactions, which may be enzymatic or microbial, will be elaborated in later chapters [21].

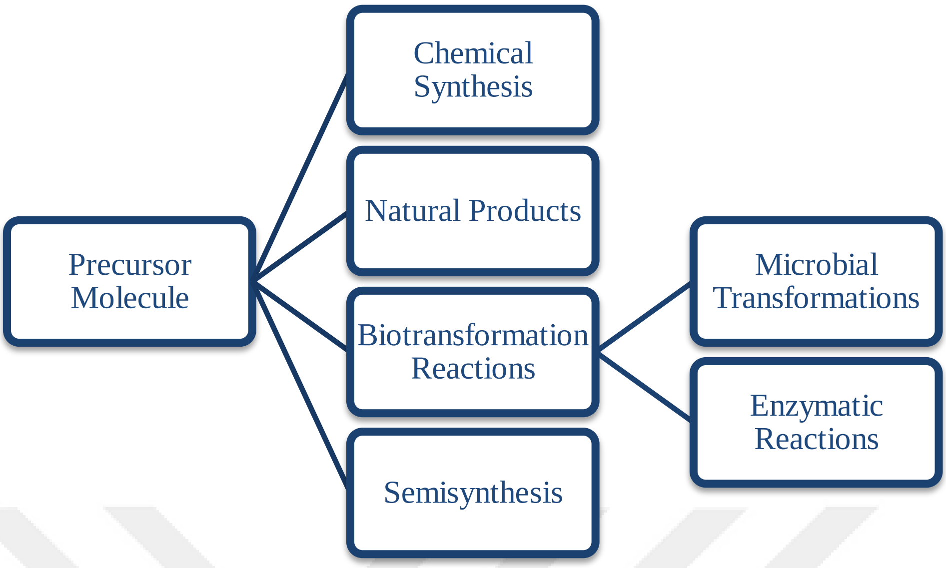


Figure 4. Drug designing from precursor molecules

1.3. Biologically Active Compounds

Biologically active compounds can be defined as compounds that can impose a direct physiological effect on animals, plants, or other microorganisms. Recently, based on their biodegradability and low toxicity, interest in biologically active compounds from natural sources has increased. Even though their functions in plants have not been yet clearly been understood, they are assumed to have protection mechanism against animals and possibly some parasites as an ecological and evolutionary advantage [22].

Biologically active compounds can be divided into 4 subgroups based on their differences in their chemical structures.

1.3.1. Terpenoids

The widest subgroup of biologically active compounds is terpenoids. The biological synthesis of terpenoids may be through two different pathways, both of which include the production of dimethylallyl diphosphate (DMAP) and isopentenyl diphosphate (IPP). Those are activated five carbon isoprene units, which have the ability of forming longer chains and undergoing modifications like oxidation or glycosylation. Terpenoids are classified based on their number of isoprene units; hemiterpenes (one unit), monoterpenes (two units), sesquiterpenes (three units), diterpenes (four units), sesterterpenes (five units), triterpenes (six units) or tetraterpenes (eight units) [23]. Biosynthesis of terpenes, sesquiterpenes has shown in the figure below.

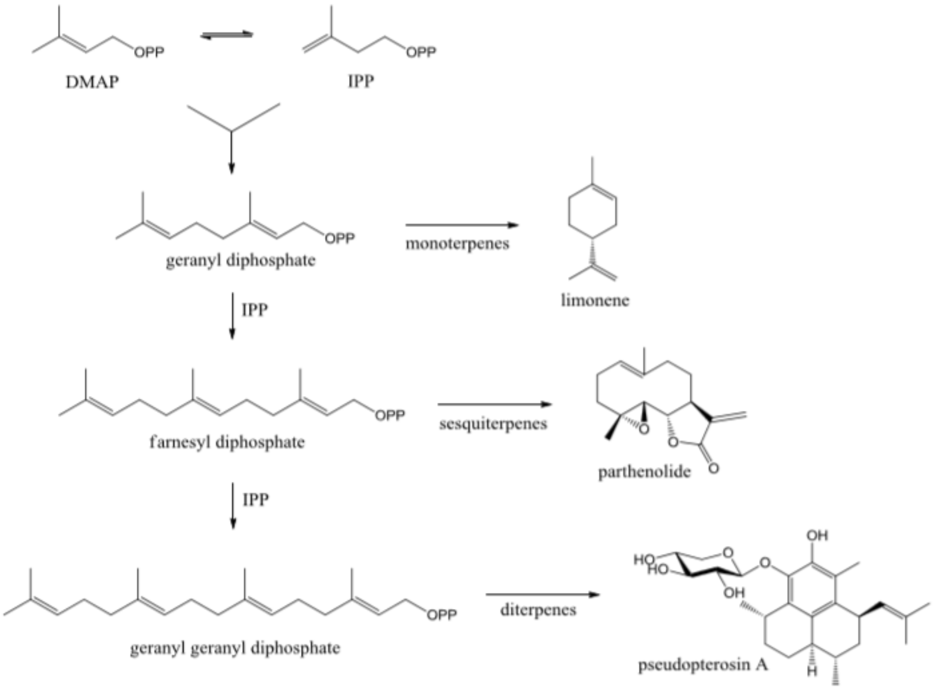


Figure 5. Biosynthesis of terpenes, sesquiterpenes and examples

1.3.2. Polyketides

Polyketides are a highly diverse group of secondary metabolites and are composed of repeating units of carbonyl and methylene groups. Due to their antimicrobial and immunosuppressive properties, they are used as cholesterol lowering agents and antimicrobial agents like tetracycline and erythromycin. They are mostly produced by fungi via polyketide syntheses enzymes [8].

1.3.3. Non-ribosomal peptides

Those compounds are mainly obtained from bacteria or fungi and they are composed of amino acids connected by peptide linkages. Interestingly, they are not produced by the ribosome, but by non-ribosomal peptide syntheases (NRPs). NRPs are very enticing candidates for novel drug research due to their proteolytic stability and selective bioactivities [24]. They are currently used as aftercare for organ transplantations, cancer therapy and as immunosuppressive agents [25].

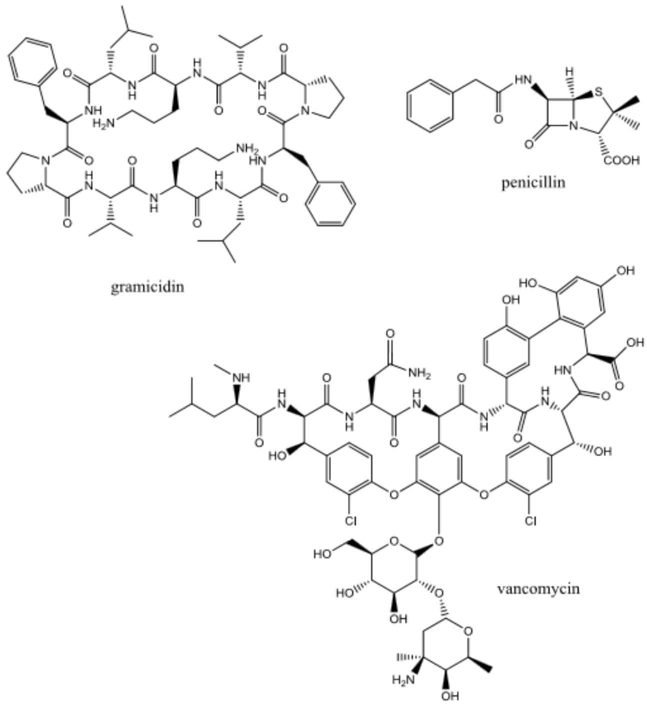


Figure 6. Examples of well-known non-ribosomal peptide antibiotics[4]

1.3.4. Alkaloids

The second biggest subgroup of biologically active compounds is alkaloids. They are derived from amino acids and known as heterocyclic compounds containing nitrogen in a negative oxidation state. Alkaloids are divided into different subgroups based on the chemical structure of their nucleus [26]. The most common amino acid building blocks and structures of alkaloids are depicted in Figure 7.

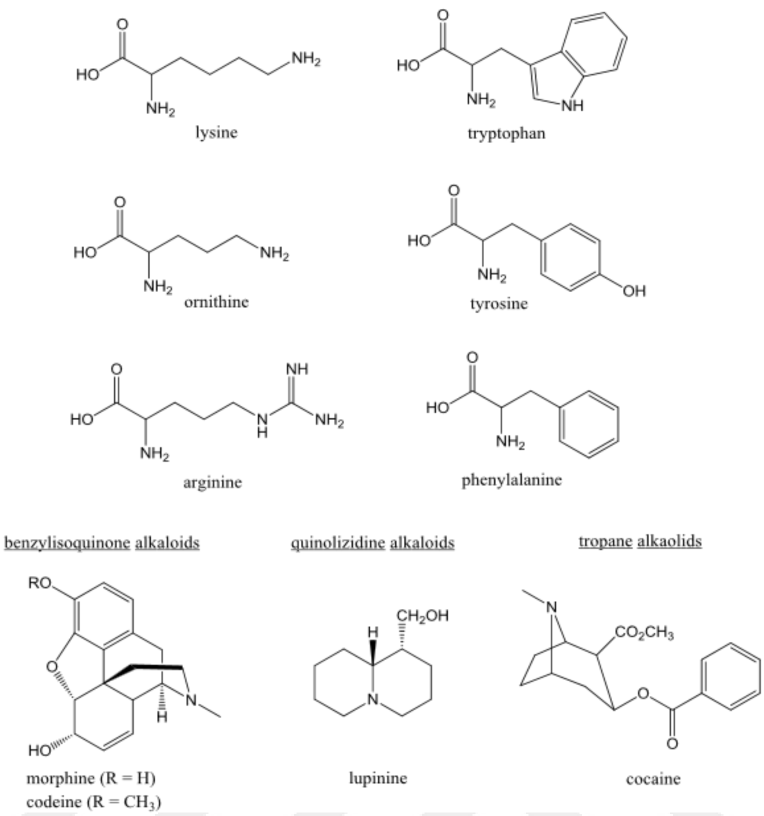


Figure 7. The most common amino acid building blocks and structures of alkaloids [4]

Alkaloids are also widely used in pharmaceutical industry due to their stimulant, analgesic, antibacterial, anticancer; antihypertensive and anti malarial properties [27]. Alkaloids are widely distributed natural compounds with highly miscellaneous metabolic functions in plants. In the light of all this information, natural products are outstanding sources of candidates to overcome the problem of rising antibacterial resistance. Research on natural products extracted from plants, on isolation of the active compounds and on evaluation and characterization of their biological activities becomes more and more important in the field of pharmacy and biochemistry.

1.3.4.1 Berberine and derivatives

Berberine was first isolated in 1826 from the Xanthoxylon cava plant. Berberine has later been extracted from *Berberidaceae*, *Papaveraceae*, and *Rutaceae* (Figure 8).

(a) (b) (c)

Figure 8. Plants containing protoberberine alkaloids: *Chelidonium majus* (a) and *Berberis vulgaris* (b, c)

The activities of berberine and it's derivatives include but are not limited to antibacterial activity against both Gram-positive and Gram-negative bacteria, antifungal activity against *Candida albicans* and *Aspergdlus fumigatus*, antiplasmodal activity against *Plasmodium falciparum*, and several other pharmacological activities such as antitumor, antiulcerous, antileukemic and enzyme inhibitory activities [28]. Figure 9 depicts the chemical structure of berberine and its derivaties.

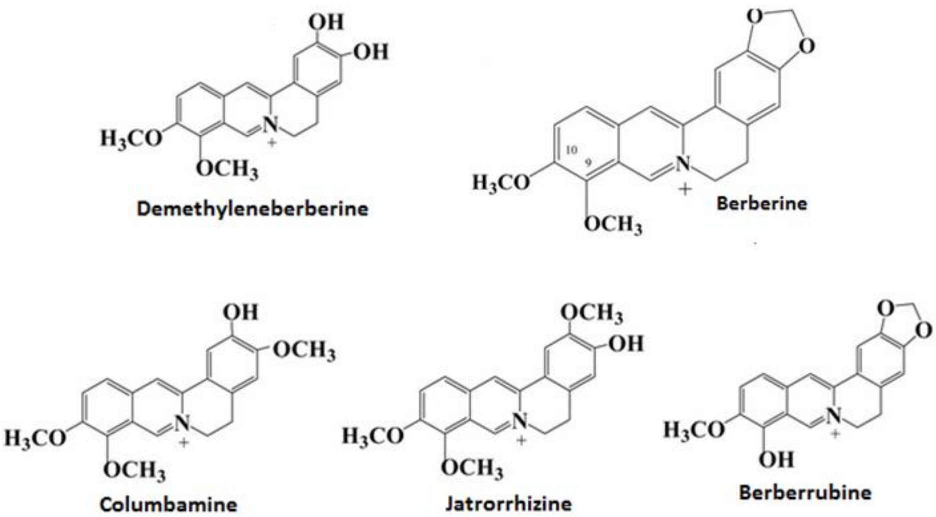


Figure 9. Chemical structure of berberine derivatives (PubChem)

There are some reports showing that berberine derivatives possess these activities due to the presence of the iminium ion moiety in the aromatic structure. This reacts with nucleophilic amino acid residues in enzymes and receptors of microorganisms.
Berberine extracts have been used for the treatment of cholera and other bacterial diarrhoea in Native American, Chinese, and Japanese traditional medicine. Among the known action mechanisms of berberine are its intercalation with DNA (Figure 10) and inhibition of reverse transcription and DNA synthesis of gram-negative bacteria [30].

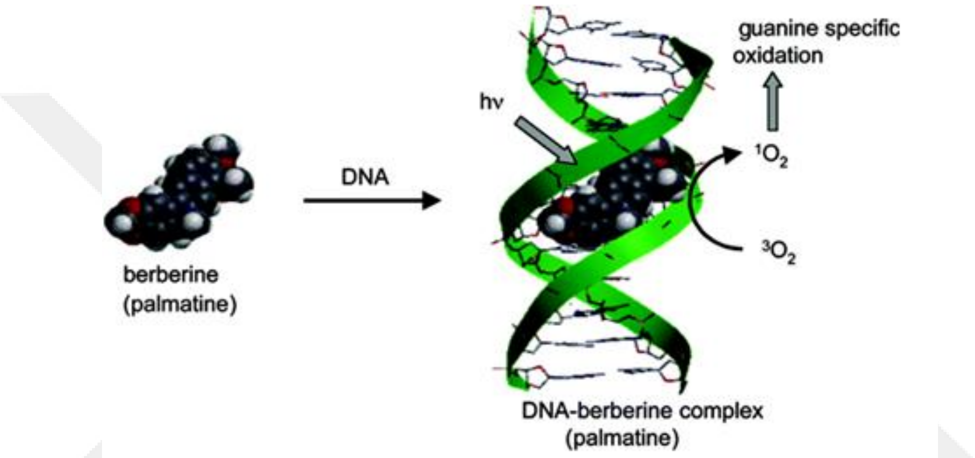


Figure 10. Action mechanism of berberine [31]

1.4. Chemical properties and activities of berberine and its derivatives

The basic skeleton of proto berberine alkaloids is 5,6-dihydrodibenzo[a,g]quinolizinium ($C_{17}H_{14}N^+$) (Figure 11).

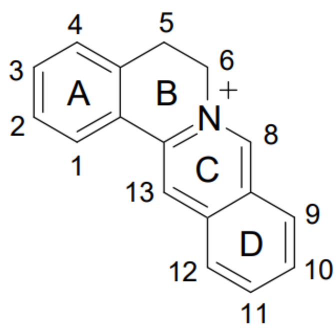
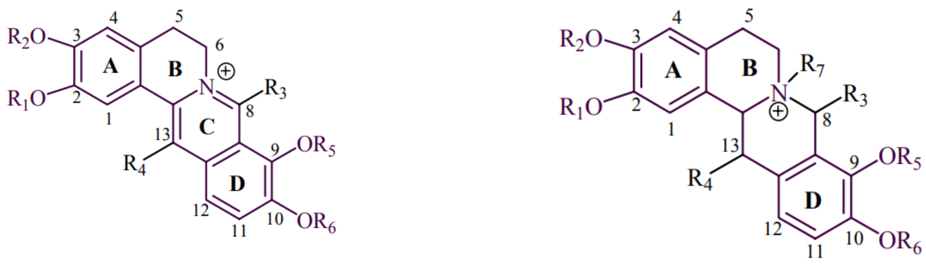


Figure 11. Basic skeleton of quaternary proto berberine alkaloids

Proto berberine most common substituents are methoxy, hydroxyl and O-CH_2-O groups. Those are mostly positioned at C 2,3,9 and 10 or at 2,3,10 and 11 [32]. The significant features for berberine's antimicrobial and cytotoxic activities are summarized below:

- *The type of O-alkyl substituent on rings A and D:* Compounds having a methylenedioxy group on ring A (where R1 and R2 = CH_2) shows a higher activity. The activities increased as the alkyl chain length increased up to dodecyl, but alkyl chains longer than the dodecyl decreases activity.
- *The presence of a quaternary nitrogen atom*
- *The Aromaticity of ring C*
- *The size of the substituent at C-13:* Compounds having alkyl side chains at C-13 shows cytotoxicity, with an increase in cytotoxicity occurring with an increasing chain length.
- *The influence of the oxygen substituents in ring A:* It has been reported that the antimalarial activity proto berberine alkaloids was influenced by the type of oxygen substituent on ring A (Figure 12a). Compounds with a methylenedioxy group on ring A (where R1 + R2 = CH_2) show higher activity against *P. falciparum* than compounds with a methoxy group at the same 4 positions, (R1 = R2 = OCH_3).

  Besides, replacement of a methoxy group at C-2 or C3 by a hydroxy group results in an increase in activity [16].



a) Berberine derivatives   b) Tetrahydroberberine derivatives

Figure 12. Basic skeleton of berberine and tetrahydroberberine derivatives

- *The influence of substituents on ring C*: There have been some publications on the effect of substituents at C-8, C-13, and of the quaternary ammonium ion of ring C on antibacterial and antimalarial activity [16]. For instance, antibacterial activity against *S. aureus* and *B. subtilis* increased with the introduction of an alkyl group at C-8 (R3 = CH_3, C_2H_5, n-C_3H_7, and n-C_4H_9) (Figure 12a) as the alkyl side chain length increased. Antimicrobial activity of these compounds also increased with the addition of a bromine atom at C-12 on ring D (Figure 12). Also, substitution of different alkyl groups at C-13 (R4, Figure12) was investigated. 13-hexylberberine derivatives were found to exhibit the highest antibacterial activity, once the alkyl side chain length was increased from a methyl to an n-hexyl group. When the R4 substituent at C-13 changed to OCOOEt, OEt, OH, OMe, and OCON(Me)2, their antimalarial activities were lower than berberine itself against *P. falciparum*. Antibacterial activity against *S. aureus* and *B. subtilis* was significantly reduced with the reduction of protoberberinium salts (Figure 12a) to tetrahydroberberine derivatives (Figure12b) [16].

- *The influence of type and position of oxygen on ring D*: The antimicrobial activity of the berberine series are strongly influenced by the oxygen group at C-9. It has been reported that berberine and berberrubine, where R5 = H (Figure 12a), both mediate the DNA cleavage; however DNA cleavage assay showed that berberrubine was more active than berberine [17]. Antibacterial activities of berberrubine against Gram-positive bacteria were increased with the C-9 alkylation of the hydroxy group of berberrubine with a haloalkane (the alkyl groups were hexyl, heptyl, octyl, nonyl, decyl, and undecyl groups) as they caused alkyl ether products.

1.5. Polymyxin and its Derivatives

Polymyxins are lipopeptide antibiotics (antibiotics which consist of acyl side chains and peptides) that contain a heptapeptide ring and three-amino acid exocyclic tails capped by a mixture of two fatty acids. They are produced by nonribosomal peptide synthetase [4]. They target the outer membrane of bacteria (Figure 13).

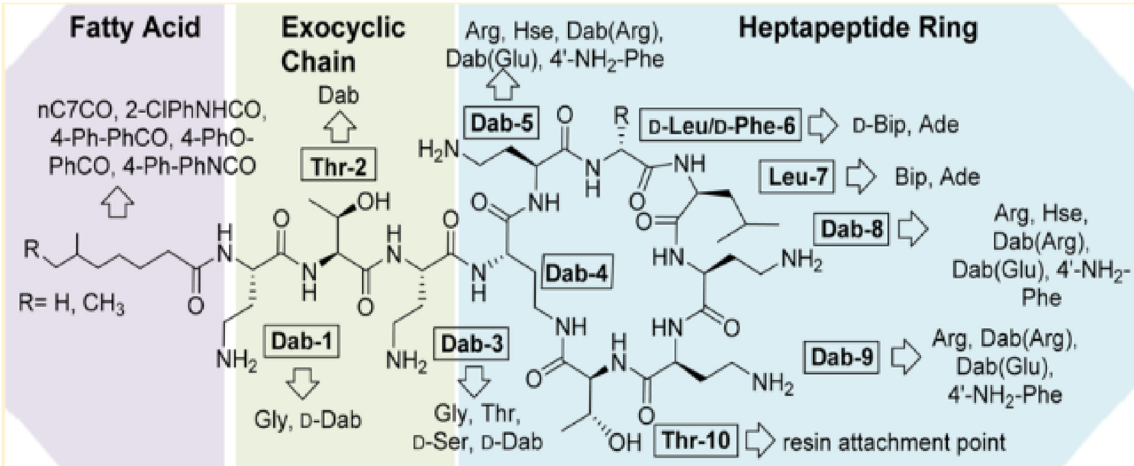


Figure 13. Molecular structure of Polymyxins [4]

Fatty acid side causes the decomposition in the hydrophobic region of cell membrane. The cyclic heptapeptide preserves optimal distances between each domain; hence provides the structure its amphipathicity. The amphipathicity has a significant effect for its antimicrobial activity.

Hydrophobic properties of the N-terminal fatty acyl segment of polymyxins are the reason for its antimicrobial activity, but this tail also results in cytotoxicity and nephrotoxicity. For this reason, polymyxins have been regarded as last resort antibiotics [33].

1.5.1. Polymyxins Mechanism of Action

The cellular outer membrane, which takes the form of a complex asymetrical structure, consists of an inner phospholipid leaflet and an outer leaflet that is mostly made up of LPS, proteins and phospolipids. LPS in its core structure contains three domains: the variable O-antigen chain (encompassing repeated saccharide units); a coreoligosaccharide region; and a conserved lipid A moiety.

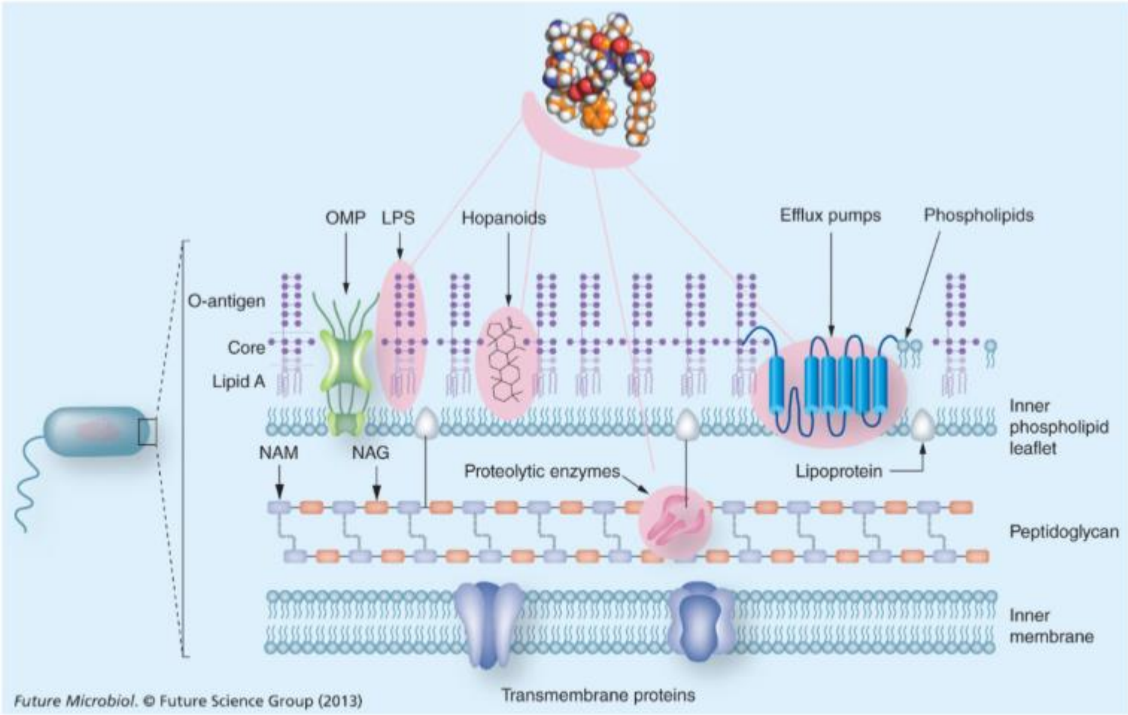


Figure 14. Key mechanisms of polymyxin resistance in Gram-negative bacteria LPS: Lipopolysaccharide; NAG: N-acetylglucosamine; NAM: N-acetylmuramic acid; OMP: Outer membrane protein [5]

Lipid A of the outer leaflet serves as a hydrophobic anchor. The saturated lipid A hydrocarbon chains are packed tightly together within the membrane by Van Der Waals forces. The highly repulsive anionic charge due to the presence of lipid A phosphor-ester moieties, O-antigen sugars and functionalities of phosphate and carboxylate within the core itself enhances the barrier function of the outer membrane.

Polymyxins can interpenetrate the outer membrane barrier by its amphipathicity. They bind to LPS and disrupt the inner and outer membranes of Gram-negative bacteria resuting in an osmotic imbalance and cell death [5].

1.6. Microbial Biotransformations

Microorganism's ability to modify certain compounds by enzymatic catalysis is called biotransformation. Biotransformation technology has become increasingly important for producing many oxidized, aromatic and aliphatic compounds for the chemical industry so that more than 130 chemicals have been recently produced via biotransformation applications. Many commercial biotransformation processes have been applied to various types of natural compounds as shown in Figure 15. Many carbohydrate and fat derivatives are mostly developed within the food sector, whereas other types are mostly find applications in pharmaceutical and agro-industries [34].

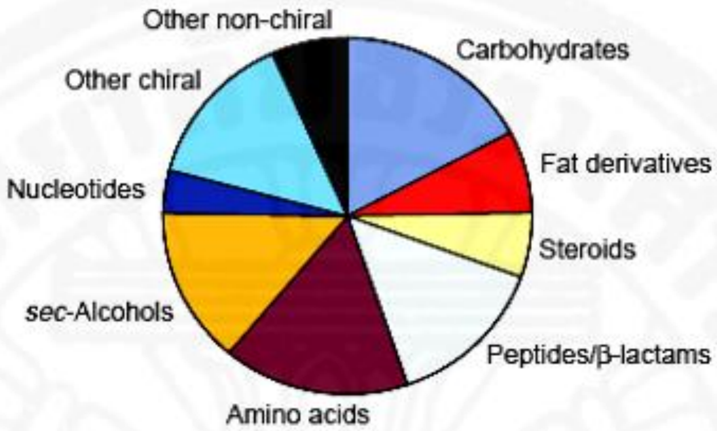


Figure 15. The type of compounds produced using biotransformation process [29]

Biotransformation can be classified according to the biocatalysts used – microbial or cell-free systems. Cell-free bio systems are based on using cell extracts or purified enzymes. Different from traditional enzymatic biotransformations that utilize one to three enzymes, cell-free bio-systems for bio-manufacturing are defined as *in vitro* bio-systems comprised of more than three enzymes in one vessel for industrial production. Cell-free bio-systems have three major distinct applications: cell-free protein synthesis (CFPS) responsible for the production of high-value proteins and antibodies, *in vitro* synthetic biology for the production of high-value drugs and fine chemicals, and synthetic enzymatic pathway biotransformation (SEPB) responsible for producing low-value bulk bio commodities, such as organic acids, hydrogen, amylose, alcohols, jet fuels, etc [35]. Biotransformation reactions and examples are shown in table 1.

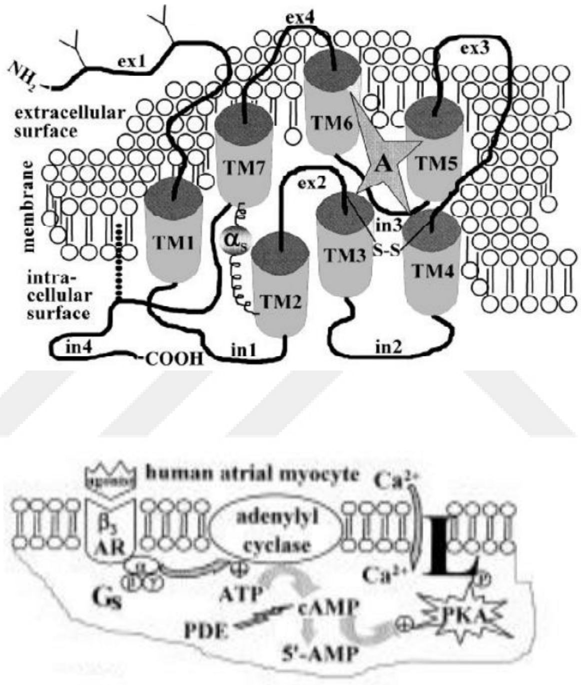
Table 1. Classification of biotransformation reactions [36]

Chemical reaction	Examples
Oxidations	Hydroxylation, epoxidation, dehydrogenation of C-C bonds. Oxidation of alcohols and aldehydes, oxidative degradation of alkyl ,carboxyalkyl or oxoalkyl chains, oxidative removal of substituents, oxidative deamination, oxidation of hetero-functions, oxidative ring fission.
Reductions	Reduction of organic acids, aldehydes, ketones and hydrogenation of C=C bonds, reduction of heterofunctions, dehydroxylation, reductive elimination of substituents
Hydrolysis	Hydrolysis o f esters, amines, amides, lactones, ethers, lactams etc. Hydration of C=C bonds and epoxides.
Condensation	Dehydration, 0- and N-acylation, glycosidation, esterification, 1actonization, amination.
Isomerization	Migration of double bonds or oxygen functions, racemization
Formation	C-C bonds or hetero-atom bonds, cation, 1actonization, amination, rearrangements

1.6.1. Cell Free Biotransformations

Enzymatic biotransformation reactions are catalyzed by enzymes, which are isolated from microorganisms or by synthetic enzymes. Enzymes are reusable and work at moderate pH and temperature values. Enzymatic biotransformations are among the preferred methods. When downstream processes of biotransformations are considered, they are much more advantageous compared to chemical synthesis or microbial biotransformation. Enzymatic biotransformation is a simple and efficient method, especially when the target molecules are obtained in more than one reaction.

There are numerous enzymatic biotransformation studies. For example, Skeberdis [37] performed biotransformation studies on developing a receptor-compatible agonist. The β3-adrenergic receptors, shown in Figure 16, are located on the cell surface of white and brown adipocytes and are responsible for lipolysis, thermogenesis and relaxation of the intestinal smooth muscles. The development of selective β3-receptor agonists for the treatment of gastrointestinal disorders, type-2 diabetes and obesity has been studied. In these studies, both isolated enzymes and microorganisms have been utilized. Figure 16 shows different results with direct enzymes and whole cells [37].



(a)

(b)

Figure 16. a) β3-adrenergic receptor b) Adipocyte location of β3-adrenergic receptors - A - β3AR agonist; αs-stimulatory subunit of G protein; TM - transmembrane domain; in - intracellular domain; ex - extracellular domain; S-S-disulphide bond; Y-N-glycosylate [37]

1.6.2. Whole Cell Biotransformations

In biotransformation studies, microbial systems are advantageous since they have the ability to produce large amounts of biomass in a short time and they can produce a wide range of chemo selective, regioselective and enantioselective enzymes. Biotransformation reactions can also be carried out chemically, of course, with everyday technology. One great advantage of whole cell biotransformations is that there is no need to add any substance to perform the reaction, especially in the protective step. The biocatalytic environment is an alternative production path that is one-way, scale-expandable and environmentally friendly [38]. The isolation and purification of enzymes required for chemical synthesis is a difficult and expensive process but, the use of whole cell as a biocatalyst is both cost effective and practical, and in many cases provides an advantage over the use of isolated enzymes. Furthermore, it is very easy to conclude cascade reactions that are encountered in the production of steroids using a single biocatalyst [39].

1.6.4. Microorganisms

For this study, the fungus *Curvularia lunata ATCC 12017* was selected. Table 2 lists the different microorganisms used for biotransformation in the literature.

Table 2. Microorganisms suitable for antibiotic biotransformation in literature review

Microorganism	Family	Ability	Reaction type	Metabolite Type	References
Curvularia lunata	Fungus	11β-hydroxylation	Cortexolone to Cortlisol	steroid hormone	[42]
Mycobacteriuns megmotis	Gram-positive bacteria	Dehydrogenation	Biotransformation of prednisolone	synthetic glucocorticoid	[43]
Vibro cholera	Gram-negative bacteria	Oxidation(including hydroxylation and ketonization)	d-limonene to terpendoits	terpenoits	[44]
Listonella damsel	Gram- negative marine bacteria				
Vibrio alginolyticus	Gram- negative marine bacteria				
Nocardia sp. NRRL 5646	Gram-positive bacteria	14β,15β-epoxy ring cleavage and acetoxylation	resibufogenin to 3-acetyl 15β-hydroxy-bufatolin		[44]
Lactobacillus farciminis	Gram-positive bacteria	Decarboxylation	Freulic acids to 4-vinyl guaiacol	volatile phenol	[45]
Aspergillus oryzae	Fungus	Hydrolysis	P.cuspidatum to resveratrol	phytoalexin	[46]
Pseudomanas spp. Strain L1B2P	Gram-negative bacteria	Oxidation	Citronellol to rose oxide	Monoterpene	[47]
Cunninghamella blakeseeana	Fungus	4-hydroxylation	Flurbiprofento 4-hydroxyflurbiprofen, 3,4-dihydroxyflurbiprofen, and 3-hydroxy,4-methoxyflurbiprofen	-	[48]
Pseudomonas aeruginosa 42A2	Gram-negative bacteria	Hydroxylation	Hydroxifatty acids: ricinoleic acid, linoleic acid, oleic acid, palmitic acid to 9,10,13-trihydroxy-11E-octadecenoic acid (9,10,13-11ETHOD) and 9,12,13-trihydroxy-10E-octadecenoic acid	Oxylipins	[49]
Streptomyces venezuelae YJ028	Gram-positive Bacteria	Hydrogenation	TrichostatinAto 2,3-dihydrotrichostatin A		[50]

Pseudomonas aeruginosa NRRL-B-18602	Gram-negative Bacteria	β-hydroxylation	Bioconverted - Eicosapentaenoic acid (bEPA)	hydroxy fatty acid	[51]
Pseudomonas aeruginosa NRRL-B-18602	Gram-negative Bacteria	β-hydroxylation	Docosahexaenoic acid to bDHA	Mono, di, tri- fatty acid	[51]
Pseudomonas aeruginosa NRRL-B-18602	Gram-negative Bacteria		Hydroxifatty acids: ricinoleic acid, linoleic acid, eicosadienoic acid, etc to bEFA	Mono, di, tri- fatty acid	[51]
Streptomyces halstedii	Gram-positive bacteria	21-hydroxylation	Galbonolides A and B to Biotransformedgalbonilides I and II		[52]
Eurtiumamstelodami Aspergillus niger	Fungus	Hydroxylation	artemisinin to 5-hydroxyartemisinin	Sesquiterpene lactone	[53]
Glomerellafusarioides ATCC 9552	Fungus	catalyze Baeyer–Villiger-type oxidation	biotransformation of Astragalussapogenins	-	[54]
Alternariaalternata NRRL2059	Fungus	Hydroxylation	α-phellandrene to 5-p-menthene-1,2-diol	Diols	[55]
Aspergillus alliaceus NRRL317	Fungus				
Aspergillus flavus	Fungus				
Botrytis cinerea AHU9424	Fungus				
Devosiariboflavina	Gram-negative bacteria				
Fusarium culmorum	Fungus				
Fusarium heterosporium	Fungus				
Fusarium solani ATCC®1284™	Fungus				
Kluyvero myceslactis	Yeast				
Neurosporacrassa	Fungus				
Phanerochaetechrysosporium	Fungus				
Saccharomyces cerevisae	Yeast				
Yarrowialipolitica	Fungus				
Cunninghamella blakeseeana	Fungus	modification of cyclopropane ring	biotransformation of Astragalussapogenins	Possible drug	[56]
Nonomurae arecticatena	Cell Free	Hydroxylation	Oleanolic Acid to Queretaroic Acid	triterpene	[57]

1.6.3 Fungal Biotransformations

Since fungi have the ability to transform readily available components to valuable metabolites, they are good candidates for chemoenzymatic synthesis of pharmaceuticals. They are already used in several industries as detoxification, methylation and hydroxylation agents due to their stereo-selective biotransformation ability. For instance; malachite green, which is used as an antifungal component, is transformed to leucomalachite green and to its N-demethylated and N-oxidized metabolites by *Cunninghamella elegans* [41]. Besides, fungi are also used in fragrance industry due to their ability of transforming terpenoids.

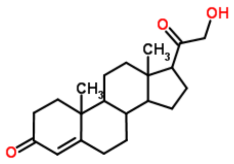
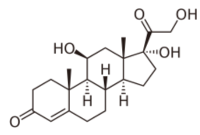
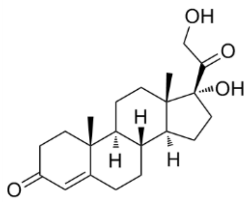
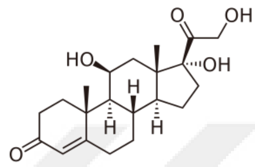
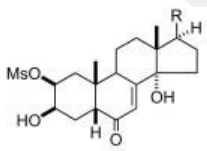
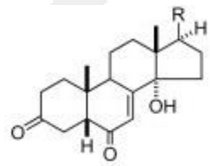
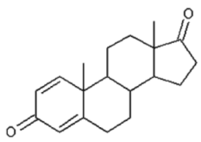
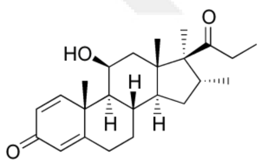
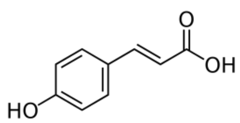
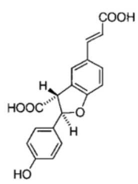
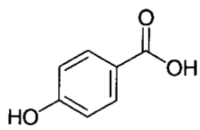
1.6.5. *Curvularia Lunata*

C. lunata is a resourceful fungus. Its conidiophores can be up to 650 µm long and 5–9 µm thick. It can be pale brown, reddish brown or dark reddish brown and it is known well for its crescent-shaped spores [59]. It is mainly used for biotransformation studies because of its ability to transform steroidal and natural substrates [58]. Due to its transformation potential, it has been studied for the development of many industrial processes [59]. The *C. lunata* enzymes involved in biotransformation processes are listed in Table 3 and the value-added or new products transformed by *C. lunata* are listed in Table 4.

Table 3. List of enzymes synthesized by *Curvularia lunata*

EC Number	Recommended Name
1.1.1.51	3(or 17)beta-hydroxysteroid dehydrogenase
1.1.1.62	17beta-estradiol 17-dehydrogenase
1.1.1.176	12alpha-hydroxysteroid dehydrogenase
1.1.1.252	tetrahydroxynaphthalene reductase
1.10.3.6	rifamycin-B oxidase
1.14.13.12	benzoate 4-monooxygenase
1.14.14.1	unspecific monooxygenase
1.14.15.4	steroid 11beta-monooxygenase
3.1.1.11	Pectinesterase

Table 4. Transformed compounds by *Curvularia lunata*[36]

Substrate	**Metabolite**
Hydroxypregn-4-en-3, 20-dione (Reichstein's substance) 	Hydrocortisone 
Cortexolone 	Hydrocortisone 
Ecdysteroid 2-mesylates 	3-Dehydro-2-deoxyecdysteroids 
16α,17α-Dimethyl-17β- (l-oxopropyl)androsta- l,4-dien- 3-one 	Rimexolone 
p-Coumaric Acid 	5-[(*E*)-2-carboxyethenyl]-2,3-dihydro-2-(4-hydroxyphenyl)-3-benzofurancarboxylic acid and 4-hydroxybenzoic acid  

1.6.6. Factors Affecting Biotransformation Process

A variety of features can affect biotransformation reactions within the concept of cost affectivity, stability and reaction yield. These parameters include the concentration of substrate – metabolites, solvent type, temperature, pH, cofactor type & concentration, and stage of growth. Some of these factors are detailed below [60].

1.6.6.1. Type of Solvents

The major obstacle of biotransformation reactions is the solubility of precursor compounds. In order to overcome this problem, usually water-soluble organic solvents such as DMF, dimethyl sulfoxide (DMSO), ethanol and methanol are used to enhance the solubility of the compound. However those solvents should not exceed 5% of the final broth volume [59].

1.6.6.2. Temperature

Usually, temperature affects both the reaction rate and the stability of the catalysts. The effect of temperature has been studied in detail for a wide range of reactions including oxidation of glycerol [61] and steroids [62] and steroid side-chain degradation [63]. For example, the transformation of rimexolone by *C. lunata* has been studied at 4 different temperatures, namely 26, 28, 30 and 32°C. The optimum temperature for the transformation by 11α-hydroxylase was at 30°C [64]. The optimal biotransformation temperature depends on the specific characteristics of the enzyme and the choice of the substrates since microorganisms may have the capability to convert each substrate at different temperatures.

1.6.6.3 Culture growth phase

The growth phase of the organism is one of the most important parameters for microbial transformation reactions. The addition of the precursors should be achieved at the right time to avoid retardation of microbial growth since usually the starting compounds such as berberine have inhibitory effects on growth [36].

CHAPTER 2

MATERIAL AND METHOD

2.1. Materials

The equipment used during the project are listed in Table 5.

Table 4. Equipment used

Name	**Supplier**
Balance	Mettler Toledo AB204-S, USA.
Spectrophotometer	PERKIN ELMER Lambda35 UV/VIS
Water Purification Systems	ELGA PURELAB, United Kingdom
Drying ovens	NÜVE FN400/500
Refrigerators	ARÇELİK, Turkey
Deepfreezer (-20˚C)	LIEBHERR, England
Deepfreezer (-80˚ C)	HETO Mini Freeze, USA
Ice Machine	Arttex Bar Line, Italia
Centrifuge	Sigma 3-18k, Germany
Micro centrifuge	Sigma 1-15k, Germany
pHmeter	Mettler Toledo MP220, USA
Horizantal Laminar Flow Cabinet	ESCO ClassII type A2, USA
Incubator Shaker	
Magnetic Stirrers	Heidolph MR3001, Germany
Vortex	Labnet, USA
Autoclave	NÜVE OT 032, Turkey
Blender	Waring, Turkey

2.1.2. Chemicals

Berberine chloride, polymyxin B, and the standard products were obtained from Sigma-Aldrich. Berberine stock solution was prepared by dissolving 100 mg of berberine chloride in 5 ml ethanol. Polymyxin B stock solution was prepared by dissolving 100 mg polymyxin-B in 2 ml H_2O.

2.1.3. Microorganism

C. lunata ATCC 12017 was purchased from the American Type Culture Collection (Manassas, Virginia, US).

2.1.4. Sterilization

Sterilized equipment was used for all experiments. All the solutions and glassware were sterilized for 15 minutes at 121˚C and 1.02 atm pressure in an autoclave. Sterilized environment was maintained under laminar flow.

2.2. Methods

2.2.1. Preparation and Maintenance of Stock Cultures

C. lunata was grown on potato dextrose agar (ATCC medium 336) (PDA) flat plates at 24°C for 7 days. The plates were stored at 4°C up to 1 month. For long-term storage, a piece of chopped agar block mycelium was placed in a sterile tube with 50% glycerol and was stored at -80ºC.

10 ml sterile water was deposited on cells in agar plates to obtain mycelium. The surface of the flat plates was scraped to get the mycelium and this was stored at -80ºC in 50% (w/v) sterile glycerol with a ratio of 1:1.

2.5. Media and cultivation

Batch growth was achieved at 24°C either in orbital shakers (114 rpm in 250 ml flasks) with vigorous aeration or in incubators without shaking for up to 15 days. Potato dextrose medium was used as the standard liquid medium. Cultivation was initiated in 250 ml erlenmeyer flasks containing 50 ml PDB. The inoculum was either an agar piece of 1x1cm chopped from a 7 days old *C. lunata* PDA plate or scraped mycelium (SS) from a PDA plate. Cultivation continued for about 14 days.

2.6. Growth Experiments on berberine containing PDA plates

To investigate the effect of berberine on the radial growth of *C. lunata*, a flamed 5mm diameter cork borer was used to cut mycelial discs from 7-day old cultures of fungi and these discs were transferred to the center of a fresh PDA plate containing different concentrations of berberine (0-0,1-0,35-0,5-1-2 mg/ml). The lowerlid of the petri dish was marked with two perpendicular lines going through the center of the plate for measurement of radial growth.

2.7. Biotransformation procedures

2.7.1. Whole-cell biotransformation

Typically, biotransformation was carried out with 50 ml fermentation media in 250 ml Erlenmeyer flasks. All media were inoculated with 7-day old *C. lunata* on 1x1cm agar. Berberine or Polymyxin-B were added to 3-day grown cells to give a final concentration of 0,35, 0,5, 1 mg/ml. Incubation continued for 14 days. In the control culture consisted *C. lunata* was grown under identical condition but without Berberine or Polymyxin-B .

2.7.2. Cell-free biotransformation process

In this method, 14-day grown cells were broken apart and the extracts were used for cell free biotransformation. To prepare the crude cell-free extract, first the cultured fungal mycelium pellets were separated using a Whatman filter. Mycelium pellets were then washed twice with 50 mM phosphate buffer with a pH of 7.

The cell cake was ground in a Waring blender in a phosphate buffer (50 mM, pH 7.0). The ground material was centrifuged at 4°C, 10000 g for 30 minutes. Supernatant was collected. Different concentrations of berberine or polymyxin-B were mixed with the supernatant. After incubation for different periods, conversion was monitored by HPLC.

2.8 Analysis methods

2.8.1. HPLC

The conversion of berberine was monitored by HPLC using a Acn:Water (10:90) applied at 0.6 ml.min-1 flow rate on a reverse phase Poroshell 120® C18-EC (50 × 4.6 mm i.d. and 2.7-µm-film thickness) column at 30 °C. 1 ml of samples collected after 0, 8th and 14th days of incubation were filtered through a sterile syringe filter with a 0.22 µm pore size and injected to HPLC.

2.8.2. Quantitative analysis of Enzyme Activity

Effect of different concentrations (0-1 mg/ml) of berberine on laccase and β-glucosidase synthesis by *C. lunata* was measured. Samples were taken after 0, 3, 7 and 14 days of incubation. Culture broth without berberine was used as control.

2.8.2.1 Laccase Enzyme Activity Assay

The fungal culture was passed through Whatman filter paper no.1 to remove mycelia and then filtered through 0.22 µm pore size filter. 1 ml samples collected after 0, 3, 7 and 14th days of incubation were assayed for activity. For laccase activity 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) –ABTS- was used as the substrate. The reaction mixture contained 950 µl acetate buffer (0.1M, pH 4.5), 200 µl ABTS (15 mM) and 50 µl sample. Properly diluted enzyme solution was incubated with the reaction mixture at room temperature for 30 minutes then absorbance was read at 420 nm. Enzyme activity was expressed in U/ml.

2.8.2.2 Beta-glucasidase Enzyme Activity Assay

The fungal culture was passed through Whatman filter paper no.1 to remove mycelia and then filtered through 0.22 µm pore size filter. 1 ml samples collected after 0, 3, 7 and 14th days of incubation were assayed for activity. For beta-glucasidase 4-Nitrophenyl β-D-glucopyranoside – pNPG- was used as the substrate. The reaction mixture contained 800 µl acetate buffer (0.1M, pH 4.5), 100 µl pNPG (10 mM) and 100 µl sample. Properly diluted enzyme solution was incubated with the reaction mixture at 45 °C for 15 minutes in a water bath. 1 ml Na_2CO_3(1M) added to the mixture to stop the reaction and absorbance was read at 420 nm. Enzyme activity was expressed in U/ml.

CHAPTER 3

RESULTS AND DISCUSSION

Polymyxin-B and berberine have been used as substrates of whole-cell and cell-free biotransformation using *C. lunata*. For this purpose, potato dextrose medium was used as the incubation medium. For whole-cell biotransformation, cells were treated with various amounts of polymyxin-B or berberine after three days of growth. Supernatants and cell extracts were assayed for biotransformation products using HPLC. A schematic representation of the biotransformation procedure is given in Figure 17.

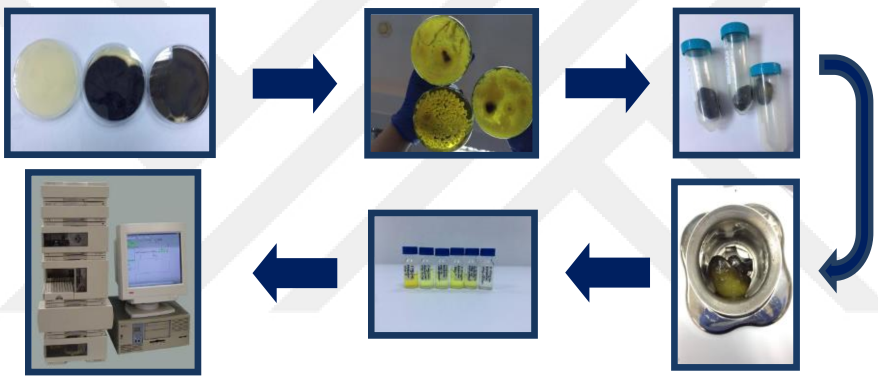


Figure 17.Schematic representation of the biotransformation procedure

3.1. Biotransformation with berberine

3.1.1. Results of Radial growth studies

Fungal growth on PDA plates containing different concentrations of berberine was carried out to determine the berberine working concentrations. When fungal growth was achieved on plates with 0.1, 0.35, 0.5, 1, and 2 mg/ml berberine, results have shown that berberine has an inhibitory effect on the growth of the fungus when its concentrations is 1 mg/ml or more concentrated. With 1 mg/ml berberine, the radial growth was very slow but with 2 mg/ml berberine, there was no growth even after 14 days of incubation (Figure 18).

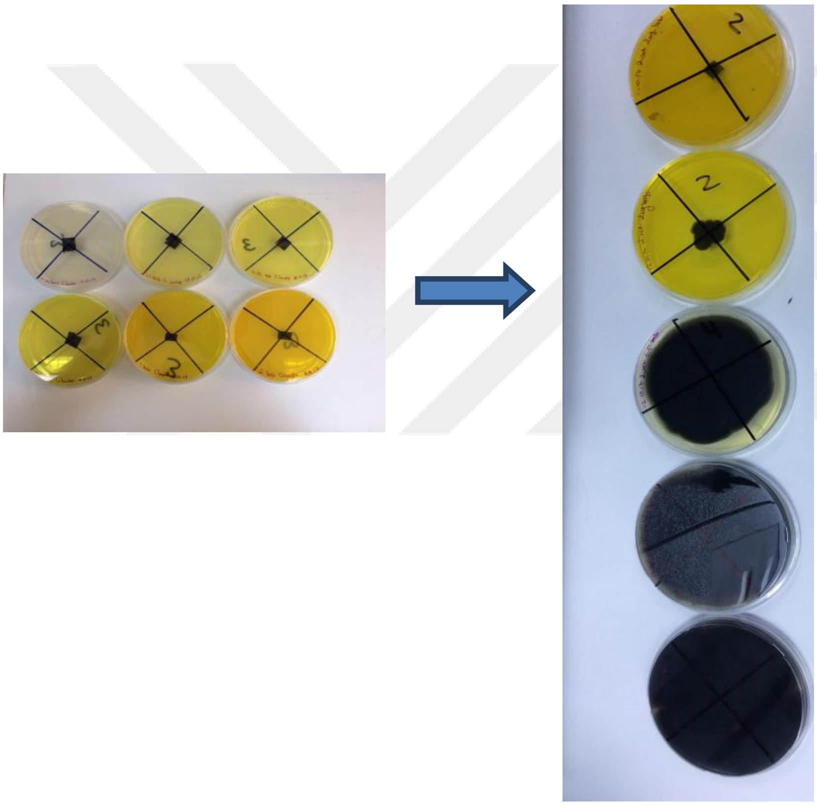


Figure 18. Radial growth experiments for 0 0.1 0.35 0.5 1 2 mg/ml berberine (lighter to dark color) for 7 days

It was also seen that with the increase in incubation period, there was an increase in the radial growth of the fungus. However, 11th day onwards, there was no substantial change in the growth rate. There were no significant difference on growth between 0 0,1 0,35 and 0,5 mg/ml berberine plates.

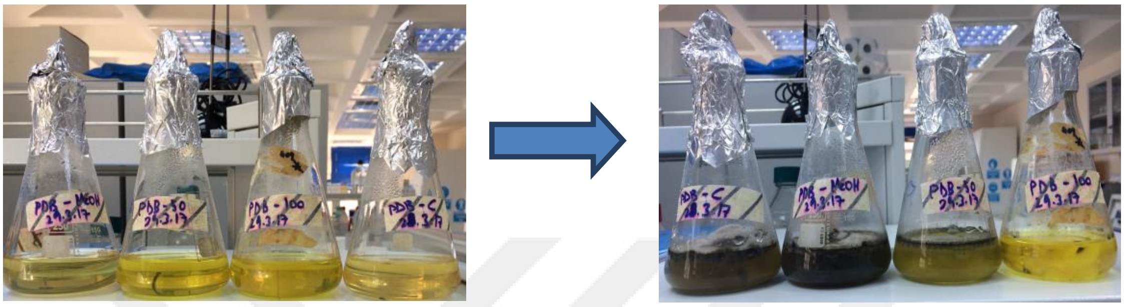


Figure 19. Stationary phase growth experiments in liquid media for 0 50 100 200 µg/ml berberine for 7 days

The radial growth results and effects of berberine on radial growth can be seen in table 5 and Figure 6.

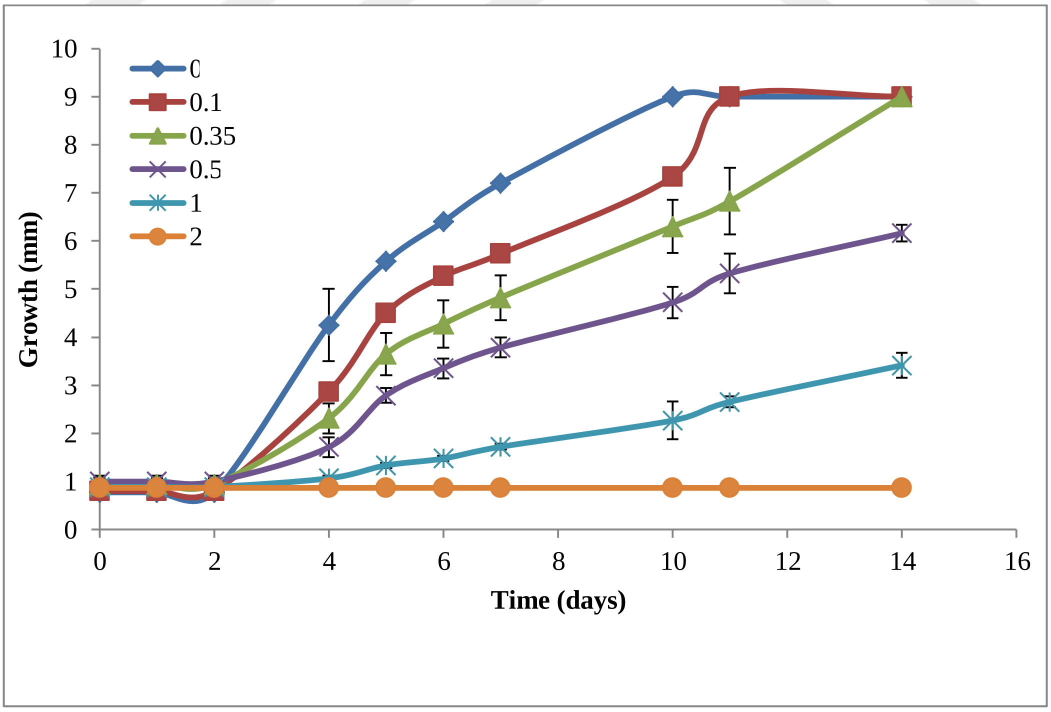


Figure 20 . Effect of different concentrations (mg/ml) of berberine on radial growth of *C. lunata*

Table 5. Radial Growth rate ($mm.day^{-}$) for each fungi cultivated in different concentrations of berberine in PDA medium.

Berberine Concentration (mg/ml)	Radial Growth Rate ($mm.day^{-}$)
0	0.818
0.1	0.724
0.35	0.634
0.5	0.464
1	0.176
2	0

Figure 18 shows that, as the concentration of berberine increased in PDA plates, the rate of growth decreased. With 0.1 and 0.35 mg/ml berberine, the growth was slower but at the end of 14 days, the whole petri was covered with *C. lunata*. On the other hand with 0.5 mg/ml berberine, growth rate dropped and the whole petri was not covered with the fungal cells after 14 days. With 1 mg/ml berberine, growth was very slow and with 2 mg/ml berberine there was no growth at all.

In order to select the range of berberine concentration for biotransformation, *C. lunata* was then grown in liquid PDB. Figure 19 shows that during stationary growth in the presence of different concentrations of berberine, as the concentrations increased sporulation became more suppressed.

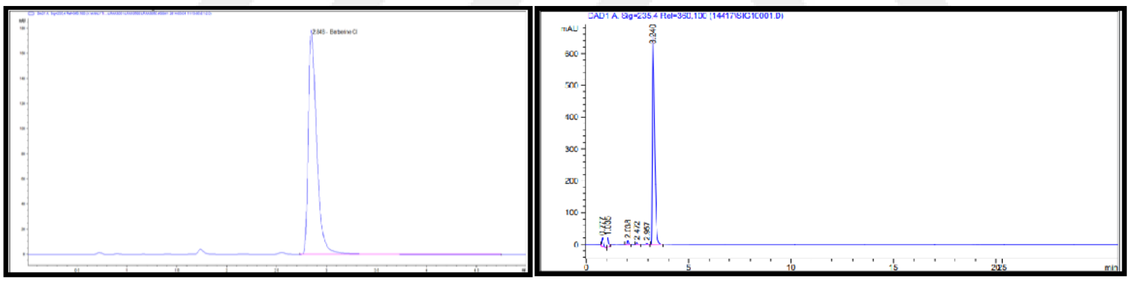
3.1.2. Determination of the time for Berberine Addition

The chemicals used in biotransformation have inhibitory effects on growth. Thus if these chemical are added to the biotransformation media at the time of inoculation, since they would prevent fungal growth, there would be no cells to perform the biotransformation. For this reason, the right time to initiate the biotransformation process was investigated.

As expected, when berberine was added to the fresh PDB together with the *C. lunata* cells, nothing grew. There was reasonable growth when berberine was introduced to the growing cells after 3 days, fungal cells continued to growth. For this reason, for the following experiments, berberine or Polymyxin B was added to 3-day grown *C. lunata* cells.

3.1.3. Quantification of Berberine

The biotransformation/biodegradation of berberine was monitored by HPLC.
To this end, a standard protocol was first established for the quantification of berberine. Various amounts of berberine were prepared and 20 µL samples were injected to the HPLC system with the mobile phase Acn:Water (10:90) at 30°C.
Figure 21 is the chromatogram obtained for the analysis of berberine chloride sulfate obtained from Sigma-Aldrich.

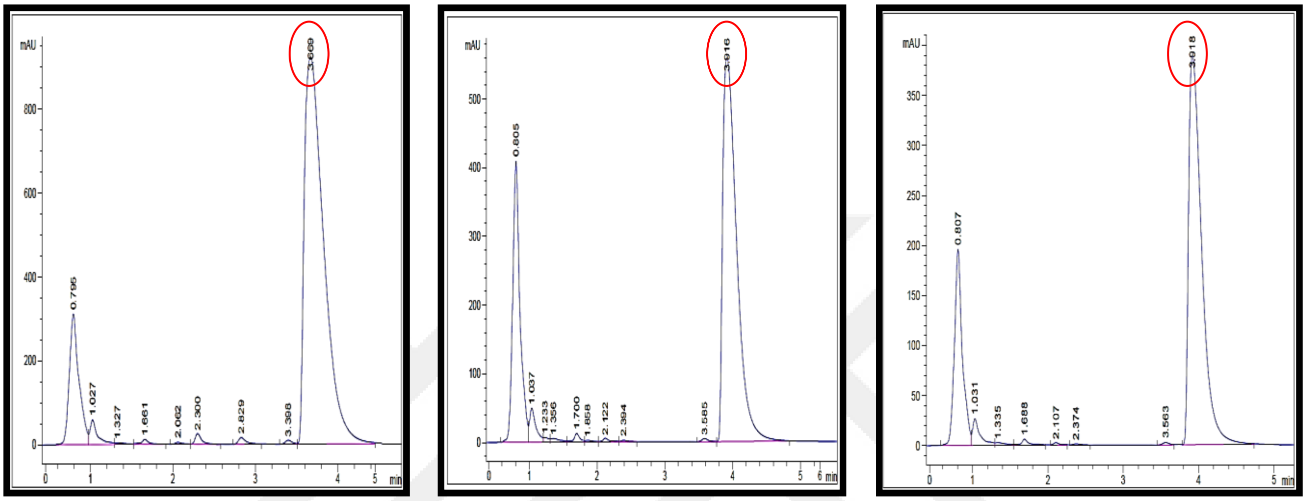


(a) (b)

Figure 21. Berberine Chloride HPLC Analysis (a) Sigma-Aldrich Certification of Analysis (b) Results of HPLC analysis of purchased Berberine Chloride

The results have shown that berberine retention time was in the range of 3.5 – 4 minutes for the reverse phase Poroshell 120® C18-EC (50 × 4.6 mm i.d. and 2.7-µm-film thickness) column. Then this procedure was used to monitor the berberine content of biotransformation reactions.

Based on the results obtained from growth experiments in the presence of berberine, two concentrations were selected for the biotransformation work: 0.35 and 0.5 mg/ml berberine. When 3-day grown cells were incubated with these selected berberine concentrations for 14 days, HPLC results (figure 22-23) have shown that berberine decreased with time especially when the initial concentration was 0.35 or 0.5 mg/ml. With 1 mg/ml berberine, cell growth and change in berberine concentration was negligible.



(a) (b) (c)

Figure 22. Chromatogram for 1 mg/ml berberine after treatment with *C. lunata* for (a) initial supernatant (b) supernatant (c) cell extract for 14 days

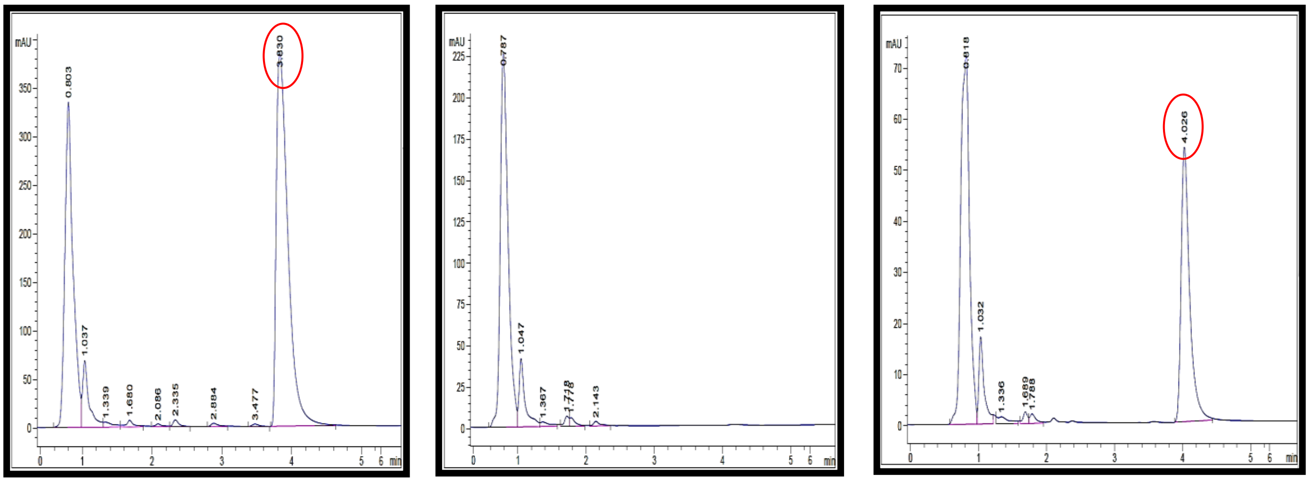


Figure 23. Chromatogram for 0.35 mg/ml berberine after treatment with *C. lunata* for (a) initial supernatant (b) supernatant (c) cell extract for 14 days.

After 14 days of incubation, 99% of the berberine in the PDB with the initial concentration of 0,35 mg/ml and 87 % berberine in the PDB with the initial concentration of 0,5 mg/ml were taken up by the cells. Only 15.7% of the 1 mg/ml of berberine was taken up by the cells after the same period. However more interestingly, although berberine was consumed by the cells, there was no other alkaloid formation in the biotransformation media, as followed by HPLC analysis and TLC.

These results indicated that *C. lunata* effectively reduced the concentration of berberine in the growth environment if present below 1 mg/ml. The intracellular accumulation of berberine was negligibly small.

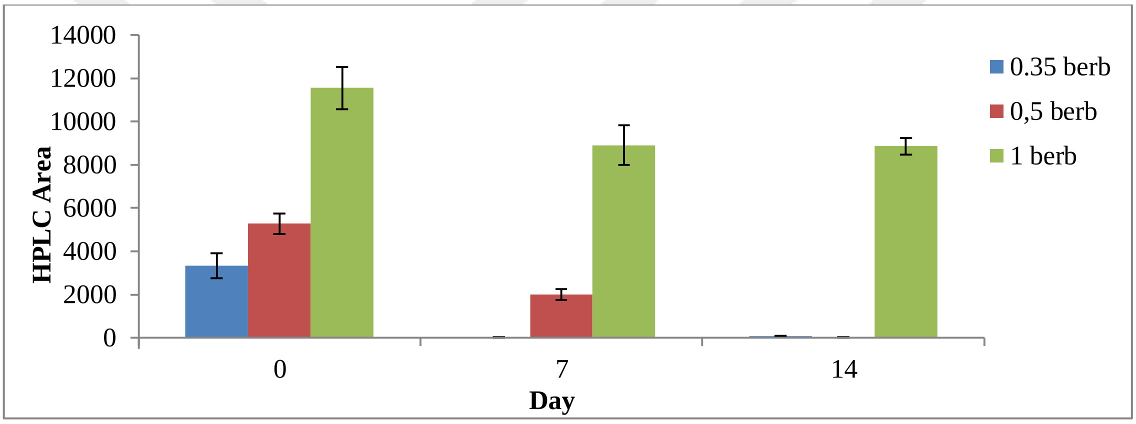


Figure 24.HPLC analyses of 0.35, 0.5 and 1 mg/ml berberine concentrations for 14 days.

Also a TLC test was performed for 14th day samples with of initial concentration 0.35 and 0.5 mg/ml berberine. The results confirmed that *C.lunata* has the ability of degradation of berberine up to 99% at those concentrations.

3.1.4. Enzyme Activity Results

After determination of berberine degradation, a literature search conducted on bio degradation of antimicrobial compounds via several fungi. Especially at Sing et. al.'s article [65], degradation of ciprofloxacin by a white rot fungus was investigated. The research group focused on the effect of ciprofloxacin on growth rate, biomass and enzyme activity. Their results shown that ciprofloxacin has a stimulatory effect on enzyme activity. A parallel research had done by getting the inspiration of Singh et. al., focusing on berberine’s effect on laccase and β-glucosidase activity.

Effect of different concentrations (0.35 0.5 and 1 mg/ml) of berberine on Laccase and β-glucasidase production of *C.lunata* was determined after 7 and 14 days of incubation. Culture broth devoid of berberine was used as control. Results can be seen in Table 6 and Figure 25.

3.1.4.1. Laccase Activity Results

It has been studied that the activity of berberine may be by either inhibiting or scavenging the ABTS radicals [58]. Berberine concentration in cultures has been monitored day by day during 14 days.

Figure 25. Laccase activity for 0.35 0.5 and 1 mg/ml berberine after treatment with *C. lunata* extract for 14 days.

We validated that at 7th day, fungi samples containing 0.35 mg/ml and 0.5 mg/ml berberine degraded berberine almost up to 100%.

Table 6. Laccase activity measurements

	Activity (U/L)		
DAY	0 mg/ml berberine	0.35 mg/ml berberine	0.5 mg/ml berberine
0	22.66±7.58	45.77±18.52	63.26±14.65
4	107.55±38.22	208.88±8.17	187.89±27.11
5	151.55±0	211.33±1.57	206.22±52.52
7	172.66±78.13	268.11±28.75	244.97±42.21
10	233.77±14.45	295.77±31.24	283.55±35.28
14	230.44±91.78	282.66±45.43	256±54.07

Laccase activities were calculated with the help of the equation below.

$$U/L = (\Delta E \times Vt)/(\varepsilon \times d \times Vs)$$

U: enzymatic units
ΔE: the change in extinction of light [min^{-1}]
ε: molar absorption coefficient of ABTS [$36000 M^{-1}\ cm^{-1}$]
d: layer thickness [cm] (1 cm)
Vt: total volume (1,2 ml)
Vs: volume of the enzyme stock solution (0,05 ml)

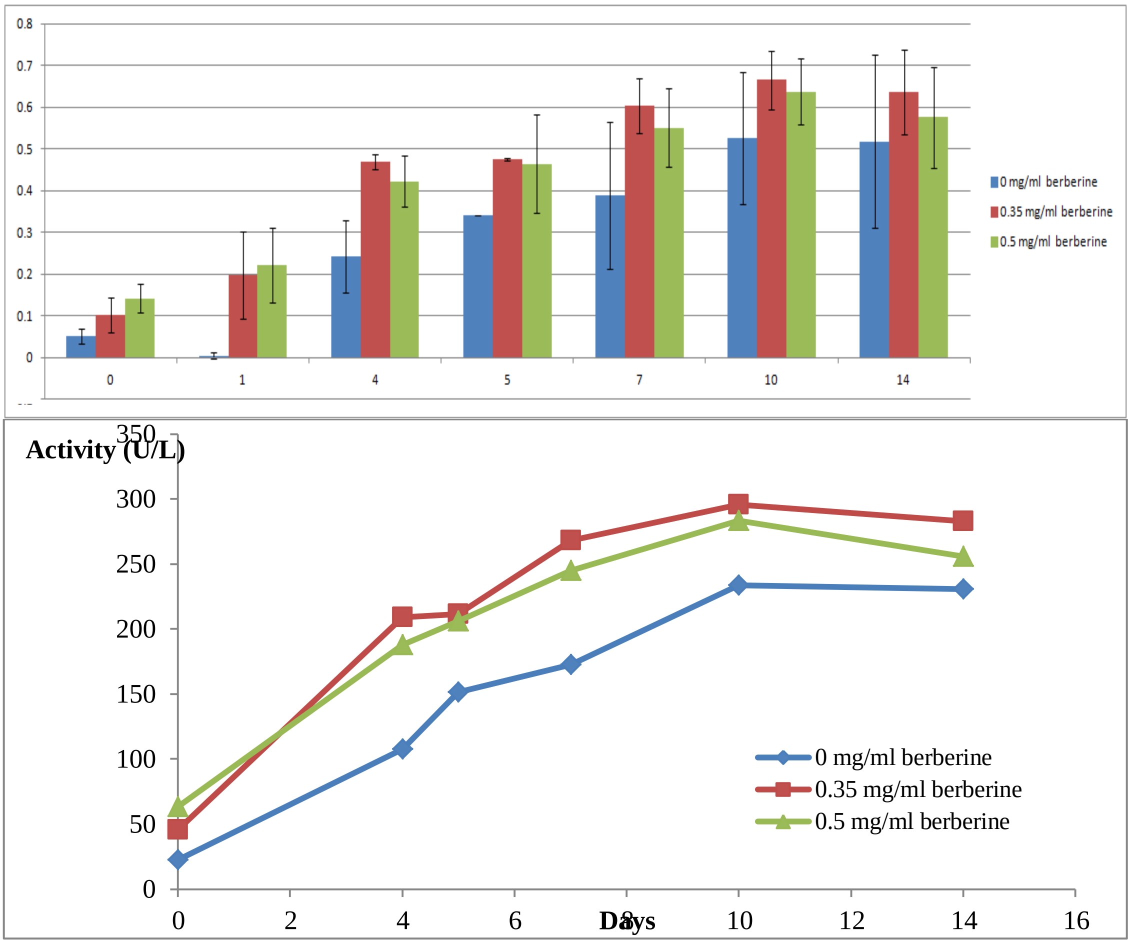


Figure 26.Effect of berberine on Laccase activity

Based on the results, there is no significant change in laccase production with different concentrations of berberine. However, sample with 0.5 mg/ml berberine, even though the growth rate decreased up to 50%, the laccase production is relatively higher than other samples.

That might indicate that laccase plays an active role in berberine degradation.

3.1.4.2. B-Glucosidase Enzyme Activity Results

Our results indicate that there is no significant change on extracellular β-glucosidase activity of berberine up to 0.5 mg/ml concentration. However 0.5 mg/ml berberine is affecting the intracellular activity of β-glucosidase.

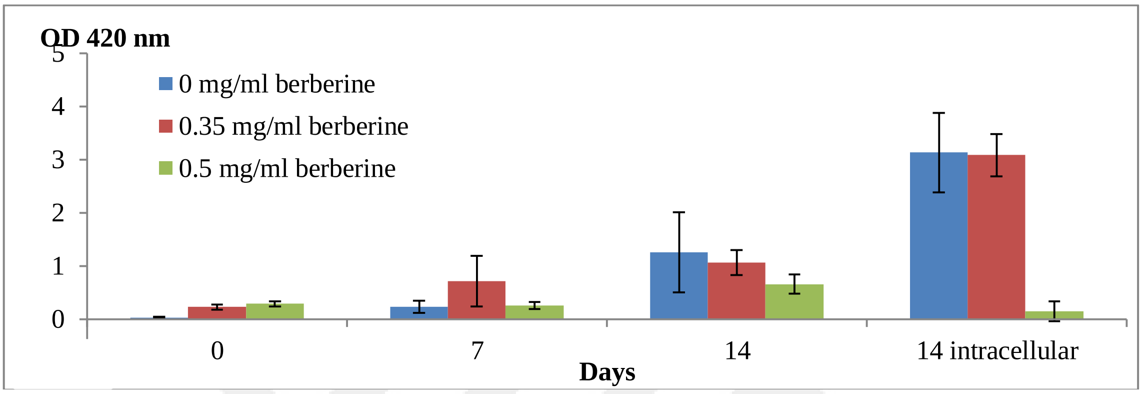


Figure 27. Effect of berberine on β-glucosidase activity

Table 7. β-Glucasidase activity measurements

	Activity (U/L)		
Day	0 mg/ml berberine	0.35 mg/ml berberine	0.5 mg/ml berberine
0	5.89±16.45	168.2±35.46	211.17±35.42
7	117±86.79	524.49±353.162	172.98±45.08
14	924.49±552.72	784.162±171.38	482.51±131.41
14 intracellular	2304.72±552.72	2269.32±291.12	44.01±20.98

Activities were calculated with the equation below:

U/ml = (ΔA*V*DF)/(ε*d*t*v)

ΔA = Absorbance change at 420 nm

t= Reaction time (15 min)

ε = Extinction coefficient of substrate (at 420 nm; 18,1)

d= Light path (1 cm)

V = total reaction volume (0,1 ml)

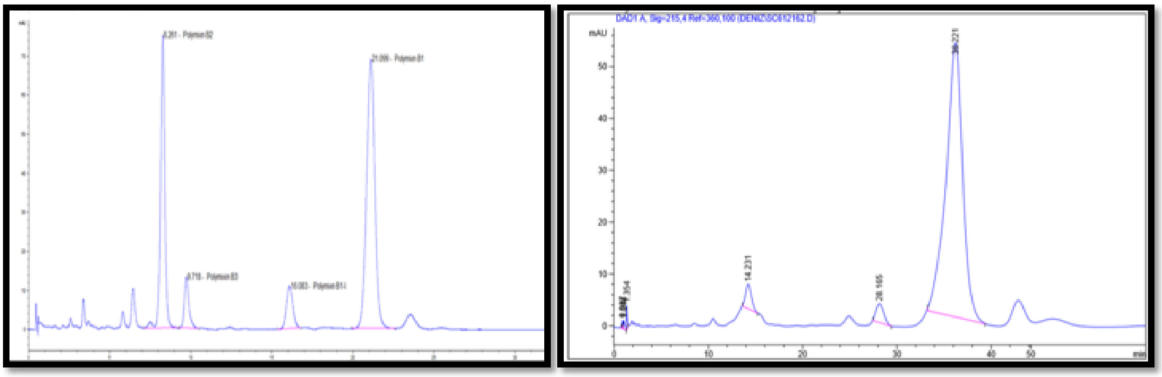
v = enzyme volume 1mM (final)

DF= dilution factor 20

3.2. Biotransformation Studies with Polymyxin B

Various amounts of polymyxin B sulfate were prepared and 20 µL sample injected to HPLC system with the mobile phase consists of Na_2SO_4 (4.5g/L) in dH_2O (pH=3.2), Acn(82:18) at the flow rate of 1 mL/min, at 30 ˚C with 215 nm wave length.

The 4 component system of Polymyxin B Sulfate has shown in Figure 28.



(a) (b)

Figure 28. Polymyxin B Sulfate HPLC Analysis (a) Sigma-Aldrich Certification of Analysis (b) Results of HPLC analysis of purchased Polymyxin B Sulfate

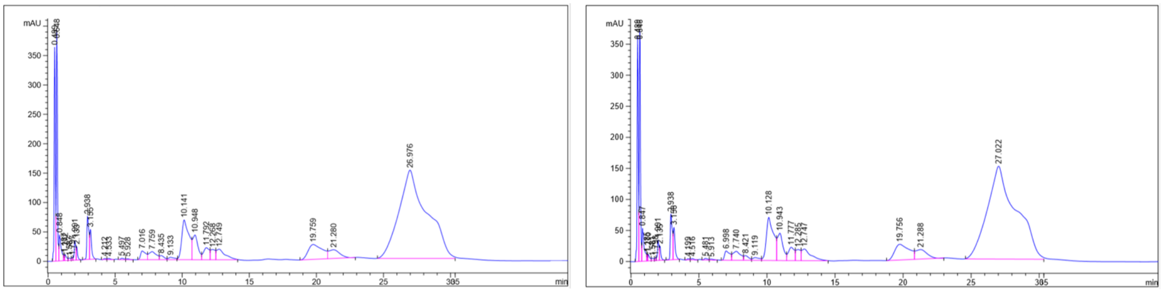
Table 9 shows the component and retention times of compounds and areas of Polymyxin B Sulfate.

Table 8. Validation of Polymyxin B Sulfate Components

	Component	Retention Time	Percentage
Reference Values	**B2**	8.261	26.60%
	B3	9.718	4.20%
	B1-I	16.038	5.90%
	B1	21.099	49.00%
	Total Percentage		**85.70%**
Measured Values	Component	Retention Time	Percentage
	B2	14.117	13.15%
	B3	16.073	6.69%
	B1-I	26.058	11.59%
	B1	37.738	56.19%
	Total Percentage		87.62%

After identification of compounds, biotransformation of polymyxin B was tested with the initial concentrations of 1 mg/ml and 2 mg/ml polymyxin B sulfate.

The complexity of media, fungal products and the small area difference between initial day and 5th day samples indicated that Curvularia lunata has no significant efficiency of transforming polymyxin B compounds.



(a) (b)

Figure 29. Polymyxin transformation experiment results (a) HPLC analysis of media consisting 1 mg/ml Polymyxin B sulfate at day 0, (b) HPLC analysis of media consisting 1 mg/ml Polymyxin B sulfate at day 5.

CHAPTER 4

Concluding remarks and future perspectives

In this thesis, we investigated the antifungal action of berberine against *Curvularia lunata* and evaluated berberine degradation for limited impact on nature. Our results indicated that *C. lunata* have a high tolerance to berberine and has a slight stimulatory effect on the laccase activity. Therefore, we showed that biotransformation via degradative fungi can be an effective mean of producing valuable compounds.

For future work, investigating berberine degradation pathway with the key enzymes can play a crucial role for providing novel antimicrobial compounds and new valuable products.

.

References

[1] WHO, “Antimicrobial resistance. Global report on surveillance,” *World Heal. Organ.*, vol. 61, no. 3, pp. 383–394, 2014.

[2] J. M. Munita, C. A. Arias, A. R. Unit, and A. De Santiago, “HHS Public Access,” 2016.

[3] and A. F. L. Benedict, R. G., “Antibiotic activity of Bacillus polymyxa,” *J. Bacteriol*, vol. 54, no. 1, p. 24, 1947.

[4] A. Gallardo-Godoy *et al.*, “Activity and Predicted Nephrotoxicity of Synthetic Antibiotics Based on Polymyxin B,” *J. Med. Chem.*, vol. 59, no. 3, pp. 1068–1077, 2016.

[5] T. Velkov, Kade D Roberts K.D.,, Nation R. L., Thompson P.E., “Antibiotics,” *Futur. Microbiol*, vol. 8, no. 6, pp. 1–20, 2014.

[6] T. R. Sampson, X. Liu, M. R. Schroeder, C. S. Kraft, E. M. Burd, and D. S. Weiss, “Rapid killing of Acinetobacter baumannii by polymyxins is mediated by a hydroxyl radical death pathway,” *Antimicrob. Agents Chemother.*, vol. 56, no. 11, pp. 5642–5649, 2012.

[7] M. P. Mingeot-Leclercq, P. M. Tulkens, S. Denamur, T. Vaara, and M. Vaara, “Novel polymyxin derivatives are less cytotoxic than polymyxin B to renal proximal tubular cells,” *Peptides*, vol. 35, no. 2, pp. 248–252, 2012.

[8] A. Sproule, “Discovery of Cryptic Microbial Natural Products Using Induction Strategies and Characterization of a Novel Biotransformation Product,” 2016.

[9] I. Pervaiz, S. Ahmad, M. A. Madni, H. Ahmad, and F. H. Khaliq, “Microbial Biotransformation: a Tool for Drug Designing (Review),” *Прикладная Биохимия И Микробиология*, vol. 49, no. 5, pp. 435–449, 2013.

[10] D. A. Rathbone and N. C. Bruce, “Microbial transformation of alkaloids,” *Curr. Opin. Microbiol.*, vol. 5, no. 3, pp. 274–281, 2002.

[11] T. Hudlicky and J. W. Reed, “Applications of biotransformations and biocatalysis to complexity generation in organic synthesis,” *Chem. Soc. Rev.*, vol. 38, no. 11, pp. 3117–3132, 2009.

[12] J. A. Rollin, T. K. Tam, and Y. H. P. Zhang, “New biotechnology paradigm: Cell-free biosystems for biomanufacturing,” *Green Chem.*, vol. 15, no. 7, pp. 1708–1719, 2013.

[13] R. K. Venisetty and V. Ciddi, “Application of Microbial Biotransformation for the New Drug Discovery Using Natural Drugs as Substrates,” pp. 1–15, 2010.

[14] H. C. Neu, “The crisis in Antibiotic resistance BT - Science,” vol. 257, pp. 1064–1073, 1992.

[15] J. Davies and D. Davies, “Origins and Evolution of Antibiotic Resistance,” *Microbiol. Mol. Biol. Rev.*, vol. 74, no. 3, pp. 417–433, 2010.

[16] J. O’Neill, “Review on Antimicrobial Resistance. Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations, 2014,” vol. 4, no. December, 2014.

[17] L. L. Founou, R. C. Founou, and S. Y. Essack, “Antibiotic resistance in the food

chain: A developing country-perspective," *Front. Microbiol.*, vol. 7, no. NOV, pp. 1–19, 2016.

[18] P. Courvalin, "Antimicrobial drug resistance: 'Prediction is very difficult, especially about the future,'" *Emerg. Infect. Dis.*, vol. 11, no. 10, pp. 1503–1506, 2005.

[19] Encyclopædia Britannica, "Antibiotic resistance," *Encyclopædia Britannica*. 2016.

[20] N. C. Cohen, "The Molecular Modeling Perspective in Drug Design," *Guideb. Mol. Model. Drug Des.*, pp. 1–17, 1996.

[21] S. Riva, *Biocatalytic Modification of Natural Products, Current Opinion in Chemical Biology*, 5th ed. 2001.

[22] A. R. War *et al.*, "Mechanisms of plant defense against insect herbivores," *Plant Signal. Behav.*, vol. 7, no. 10, pp. 1306–1320, 2012.

[23] T. Kuzuyama, "Biosynthetic studies on terpenoids produced by Streptomyces," *J. Antibiot. (Tokyo).*, vol. 70, no. 7, pp. 811–818, 2017.

[24] E. A. Felnagle *et al.*, "NIH Public Access," *Mol Pharm*, vol. 5, no. 2, pp. 191–211, 2011.

[25] M. A. Martínez-Núñez and V. E. L. y López, "Nonribosomal peptides synthetases and their applications in industry," *Sustain. Chem. Process.*, vol. 4, no. 1, p. 13, 2016.

[26] J. Buckingham, *Dictionary of Alkaloids*, 2nd ed. Boca Raton, FL: CRC Press, 2010.

[27] V. Kuete, *Health Effects of Alkaloids from African Medicinal Plants*. Elsevier Inc., 2014.

[28] A. H. Amin, T. V. Subbaiah, and K. M. Abbasi, "Berberine sulfate: antimicrobial activity, bioassay, and mode of action," *Can. J. Microbiol.*, vol. 15, no. 9, pp. 1067–1076, 1969.

[29] R. Verpoorte, A. Contin, and J. Memelink, "Biotechnology for the production of plant secondary metabolites," *Phytochem. Rev.*, vol. 1, no. 1, pp. 13–25, 2002.

[30] N. Turner *et al.*, "Berberine and Its More Biologically Available Derivative Dihyroberberine, Inhibit Mitochondrial Respiration," *Diabetes*, vol. 57, no. May, pp. 1414–1418, 2008.

[31] K. Hirakawa, S. Kawanishi, and T. Hirano, "The mechanism of guanine specific photooxidation in the presence of berberine and palmatine: Activation of photosensitized singlet oxygen generation through DNA-binding interaction," *Chem. Res. Toxicol.*, vol. 18, no. 10, pp. 1545–1552, 2005.

[32] C.W.W. Beecher and W.J. Kelleher, "Enzymatic Study Of The Late Stages Of Protoberberine Alkaloid Biosynthesis," vol. 25, no. 4595, 1984.

[33] P. Poirel, L., Jayol, A., Nordmann, "Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes," *Am. Soc. Microbiol.*, vol. 30, no. 2, pp. 557–596, 2017.

[34] R. de C. M. d. Miranda, E. de B. Gomes, N. Pereira, M. A. Marin-Morales, K. M. G. Machado, and N. B. de Gusmão, "Biotreatment of textile effluent in static bioreactor by Curvularia lunata URM 6179 and Phanerochaete chrysosporium

URM 6181," *Bioresour. Technol.*, vol. 142, pp. 361–367, 2013.

[35] C. C. C. R. Carvalho, "Whole cell biocatalysts: essential workers from Nature to the industry," *Microb. Biotechnol.*, vol. 10, no. 2, pp. 250–263, 2017.

[36] H. G. W. Leuenberger, "International Union of Pure Biotransforimation-a Useful Tool," *Pure Appl. Chem.*, vol. 62, no. 4, pp. 753–768, 1990.

[37] V. A. Skeberdis, "Ap Ž Valginiai Straipsniai," vol. 40, no. 5, 2004.

[38] M. H. P. Leresche, J. E., *No TitlChemocatalysis and Biocatalysis (Biotransformation): Some Thoughts of a Chemist and of a Biotechnologist, Organic Process Research & Developmente*, 10th ed. 2006.

[39] M. M. R. D. F. Carla C.C.R. de Carvalho, *Biotransformation of terpenes, Biotechnology Advances*, 24th ed. 2006.

[40] X. He *et al.*, "Microbial metabolism of methyl protodioscin by Aspergillus niger culture-A new androstenedione producing way from steroid," *J. Steroid Biochem. Mol. Biol.*, vol. 100, no. 1–3, pp. 87–94, 2006.

[41] I. D. Haggblom, M. M; Bossert, *Dehalogenation: Microbial Proscesses and Environmental Applications*. Springer publication, 2003.

[42] K. Paraszkiewicz and J. Długon, "Cortexolone 11 i -hydroxylation in protoplasts of Cur 6 ularia lunata," vol. 65, pp. 217–224, 1998.

[43] M. Bredehöft, R. Baginski, M. K. Parr, M. Thevis, and W. Schänzer, "Investigations of the microbial transformation of cortisol to prednisolone in urine samples," *J. Steroid Biochem. Mol. Biol.*, vol. 129, no. 1–2, pp. 54–60, 2012.

[44] H. Li, W. Lan, C. Cai, Y. Zhou, and Y. Lin, "Biotransformation of Limonene by Marine Bacteria," *Chinese J. Anal. Chem.*, vol. 34, no. 7, pp. 946–950, 2006.

[45] A. A. Hadiza, I. Shahid, W. C. Kim, and I. Maznah, "Biotransformation of ferulic acid to 4-vinyl guaiacol by Lactobacillus farciminis," *African J. Biotechnol.*, vol. 11, no. 5, pp. 1177–1184, 2012.

[46] Y. Z. Mei, R. X. Liu, D. P. Wang, X. Wang, and C. C. Dai, "Biocatalysis and biotransformation of resveratrol in microorganisms," *Biotechnol. Lett.*, vol. 37, no. 1, pp. 9–18, 2015.

[47] M. R. Pimentel, G. Molina, T. C. P. Bertucci, and G. M. Pastore, "Biotransformation of citronellol in rose oxide by pseudomonas spp.," *Chem. Eng. Trans.*, vol. 27, no. 1960, pp. 295–300, 2012.

[48] J. Amadio and C. D. Murphy, "Biotransformation of fluorobiphenyl by Cunninghamella elegans," *Appl. Microbiol. Biotechnol.*, vol. 86, no. 1, pp. 345–351, 2010.

[49] I. Martin-Arjol, M. Bassas-Galia, E. Bermudo, F. Garcia, and A. Manresa, "Identification of oxylipins with antifungal activity by LC-MS/MS from the supernatant of Pseudomonas 42A2," *Chem. Phys. Lipids*, vol. 163, no. 4–5, pp. 341–346, 2010.

[50] S. R. Park, M. S. Ahn, A. R. Han, J. W. Park, and Y. J. Yoon, "Enhanced flavonoid production in Streptomyces venezuelae via metabolic engineering," *J. Microbiol. Biotechnol.*, vol. 21, no. 11, pp. 1143–1146, 2011.

[51] V. K. Bajpai, S. Y. Shin, H. R. Kim, and S. C. Kang, "Anti-fungal action of

bioconverted eicosapentaenoic acid (bEPA) against plant pathogens," *Ind. Crops Prod.*, vol. 27, no. 1, pp. 136–141, 2008.

[52] G. H. Harris, J. M. Sigmund, J. Mark, S. Miller, N. Y. Academy, and V. Pp, "United States Patent (19) 11 Patent Number : 75 Inventors : Ali Alasoft," no. 19, 1999.

[53] I. A. Parshikov, K. M. Muraleedharan, M. A. Avery, and J. S. Williamson, "Transformation of artemisinin by Cunninghamella elegans," *Appl. Microbiol. Biotechnol.*, vol. 64, no. 6, pp. 782–786, 2004.

[54] I. Çalis, H. A. Gazar, S. Piacente, and C. Pizza, "Secondary metabolites from the roots of Astragalus zahlbruckneri," *J. Nat. Prod.*, vol. 64, no. 9, pp. 1179–1182, 2001.

[55] G. Işcan, N. Kirimer, F. Demirci, B. Demirci, Y. Noma, and K. H. C. Başer, "Biotransformation of (-)-(R)-α-phellandrene: Antimicrobial activity of its major metabolite," *Chem. Biodivers.*, vol. 9, no. 8, pp. 1525–1532, 2012.

[56] E. Bedir, C. Kula, Ö. Öner, M. Altaş, Ö. Taı, and G. Öngen, "Microbial transformation of Astragalus sapogenins using Cunninghamella blakesleeana NRRL 1369 and Glomerella fusarioides ATCC 9552," *J. Mol. Catal. B Enzym.*, vol. 115, pp. 29–34, 2015.

[57] Y. Fujii, S. Hirosue, T. Fujii, N. Matsumoto, H. Agematu, and A. Arisawa, "Hydroxylation of oleanolic acid to queretaroic acid by cytochrome P450 from Nonomuraea recticatena.," *Biosci. Biotechnol. Biochem.*, vol. 70, no. 9, pp. 2299–2302, 2006.

[58] R. M. Vohra, U. C. Banerjee, S. Das, and S. Dube, "Microbial transformation of rifamycin B: A new extracellular oxidase from Curvularia lunata," *Biotechnol. Lett.*, vol. 11, no. 12, pp. 851–854, 1989.

[59] J. Manosroi, M. Abe, and A. Manosroi, "Biotransformation of steroidal drugs using microorganisms screened from various sites in Chiang Mai, Thailand," *Bioresour. Technol.*, vol. 69, no. 1, pp. 67–73, 1999.

[60] R. Singh, "Microbial Biotransformation: A Process for Chemical Alterations," *J. Bacteriol. Mycol. Open Access*, vol. 4, no. 2, pp. 2–7, 2017.

[61] P. Adlercreutz, O. Holst, and B. Mattiasson, "Characterization of Gluconobacter oxydans immobilized in calcium alginate," *Appl. Microbiol. Biotechnol.*, vol. 22, no. 1, pp. 1–7, 1985.

[62] T. G. Park and A. S. Hoffman, "Immobilization of Arthrobacter simplex in Thermally Reversible Hydrogels: Effect of Gel Hydrophobicity on Steroid Conversion," *Biotechnol. Prog.*, vol. 7, no. 5, pp. 383–390, 1991.

[63] A. C. P. Dias, J. M. S. Cabral, and H. M. Pinheiro, "Sterol side-chain cleavage with immobilized Mycobacterium cells in water-immiscible organic solvents," *Enzyme Microb. Technol.*, vol. 16, no. 8, pp. 708–714, 1994.

[64] J. Yang, S. Yang, Y. L. Yang, H. Zheng, L. Weng, and L. Liu, "Microbial hydroxylation of 16α,17α-dimethyl-17β-(l-oxopropyl)androsta-l,4-dien-3-one to rimexolone by Curvularia lunata AS 3.4381," *J. Mol. Catal. B Enzym.*, vol. 47, no. 3–4, pp. 155–158, 2007.

[65] K. Singh, R. Khajuria, L. Kaur, "Biodegradation of ciprofloxacin by white rot fungus Pleurotus ostreatus," *3 Biotech.*, vol 69, no.7, pp. 1-8, 2017

1 Place Du Bouffay, 44000 Nantes
(+33 7 78 10 56 27)
deniz_yilmaz_@hotmail.com
Turkish, 28 years old

Deniz YILMAZ

Seeking a PhD Placement in Biotechnology starting from april 2019

Education

February 2019 — **Marmara University - Masters of Science in Bioengineering**.
- Master's Thesis: Biotransformation of Different Alkaloids and Antibiotics into Biologically Active Natural Products : *(Presented at BioMicroWorld, as poster presentation)*
- Investigation of cellular differentiation by integration of OMICs data : *experiments on the potentials of the plant-derived alkaloids as antibacterials in E. coli cells using microarray technologies.*
- Investigation of the effects of different media on the motility of Escherichia Coli : *(Submitted to Annals of Microbiology Journal)*

September 2014 — **Istanbul Technical University - Bachelors of Science in Chemical Engineering**.
- Graduation Project: Investigate bi-functional colloidal gold dispersion formation with a good colloidal stability and pH dependent agglomeration behavior in order to use in bio-detection applications
- Design of an electricity production facility from biomass

Experience

March–September 2018 — **Institut de Biochimie et Chimie des Proteines, CNRS, Project Internship**.
- Localization of a bacterial ABC Transporter (BmrA) of *Bacillus Subtilis* and to analyze how it influenced by the presence of antibiotics.
- Investigation of ATP dependent transport activity. ATP/GTP hydrolysis preference of Efr efflux pumps in *Enterococcus faecalis* and *Bacillus subtilis*.

September 2016- March 2018 — **Marmara University, Undergraduate Student Supervisor**.
- Supervise undergraduate students with experimental execution and data collection, maintain basic laboratory functions.

May 2015 - January 2016 — **Procter and Gamble, MEA Regional Raw Material Supply Chain Project Specialist**.
- *Conducting supply chain projects with stakeholders from internal team, customer, suppliers, 3PL.*

Publications

2017, October — - Yilmaz, D., Sayar, A., Akbulut, B. S. Biotransformation of different molecules by Curvularia Lunata. Poster session presented at the annual meeting of Environmental, Industrial and Applied Microbiology - BioMicroWorld Madrid(Spain)

2018, April — - Avci, F.G., Altinisik, F. E., Yuce, M., Atas,B., Aksoy,S., Kurpejovic,E., Yilmaz, D., Ulu, D.,V., Ulgen,K., Ozkirimli, E., Akbulut, B.S., Microfluidic technology based imaging enables monitoring of flagellar motility, Annals of Microbiology, submitted.

Laboratory skills

Process development, DNA and RNA extraction, PCR, quantitative RT-PCR, Gel electrophoresis, Gel extraction and PCR purification,Fluorometric nucleic acid quantitation, QuBit, Western blotting, High performance liquid chromatography, Bacterial culture and transformation, UV-visible absorbance spectroscopy, Thin layer chromatography, ChemiDoc MP imaging system

Languages

English - full professional proficiency

French - limited professional proficiency