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# USE OF MICROORGANISMS FOR BIOLOGICAL AND BIOTECHNOLOGICAL DRUGS DEVELOPMENT

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## ABSTRACT

Pharmaceutical biotechnology has been essential for the development of new drugs. Biological medicines consist of substances obtained from natural sources, chemical synthesis (exact copies of the molecules present in nature), or genetic modification of said sources. Additionally, biotechnological products include only those manufactured by applying genetic engineering techniques such as recombinant DNA technology, which can be divided into transformation, phage library, and non-bacterial transformation. Microorganisms play a vital role in these products. For the industrial processes associated with them, it is necessary to control multiple conditions to promote their optimal growth, such as temperature, oxygen, and pH. The studies have made it possible to obtain antineoplastics (marizomib, actinomycin D, romidepsin), antibiotics (2-allyloxyphenol, essramycin, anthracimycin, pargamicin A, tetracycline, streptomycin), immunosuppressants (cyclosporin A, rapamycin), and hormones (human recombinant insulin). Some have been commercialized, while others are in distinct research stages. Despite the success achieved, the discoveries continue, reflecting the multiple efforts to know the resources provided by nature to obtain substances with varied pharmacological effects.

**KEYWORDS:** pharmaceutical biotechnology, biological drug, biotechnological drug, bacterium, yeast, alga.

## INTRODUCTION

Biotechnology has been identified in various scientific, industrial, and commercial processes. It is defined as manipulating living organisms or components derived from them to produce products in areas such as drug development, animal and human nutrition, agricultural improvement, and environmental protection.[1]

Within this science is pharmaceutical biotechnology. It uses research findings in molecular and cell biology, biochemistry, genetics, bioinformatics, microbiology, bioprocess engineering, and separation technologies. They all allow the production of drugs.[2]

Biological products are substances obtained from natural sources (organs, tissues, fluids, and microorganisms) or by chemical synthesis, which are exact copies of those found in nature. They can also be produced naturally or by genetically modifying bacteria, yeasts, insect or mammalian cells, and plants. They range from small molecules to large constructs such as whole cells or viruses.[3, 4, 5]

Among the biological medicines are biotechnological ones,[6] which are obtained by applying genetic engineering techniques such as recombinant deoxyribonucleic acid (DNA) technology.[7, 8, 9] This knowledge makes it possible to transfer genes from one species to the genome of another and utilize the latter's metabolic machinery to produce a protein that it would not naturally synthesize.[10]

This modification is done through a vector. A vector is a vehicle, often a virus or a plasmid, that transports a DNA sequence to the desired host cell. Depending on its application, it can help replicate or express foreign DNA.[11]

Due to current needs, it is vital to understand the concepts related to how drugs are obtained with this technology, primarily through microorganisms. Proteins were acquired employing other methods for many years until insulin was the first recombinant product approved by the US Food and Drug Administration (FDA).[12] This scenario demonstrated that certain substances could be synthesized from genetically altered living systems.[3]

Microorganisms are an inexhaustible resource of bioactive metabolites with structural diversity and have given rise to essential molecules in the pharmaceutical industry.[13] Bacteria, yeasts, algae, and fungi have enabled the elaboration of products that, at the time, have revolutionized public health and the treatment of particular diseases.[4, 14, 15, 16, 17]

As a result, many medications have been approved and commercialized in the last decade. The emergence of new and/or better treatments for conditions such as cancer,[18] bacterial infections,[19] autoimmune and viral diseases,[20] and hormonal disorders[21] shows the improvement that microorganisms have contributed to people's quality of life.

Therefore, this review aims to highlight the role of microorganisms in developing biological and biotechnological medicines.

## CONDITIONS TO CONTROL FOR OPTIMAL MICROBIAL GROWTH

Microorganisms and the control of their growth are necessary to manufacture new drugs. The inhibition or promotion of its development is related to obtaining a metabolite with a specific therapeutic effect in humans.[22, 23]

This control is affected by different factors influencing the process's speed and efficiency. Temperature is the most important environmental issue, as it increases the rates of chemical and enzymatic reactions. However, protein denaturation will occur above a given value, affecting the microorganisms' functioning. Therefore, according to the optimum growth temperature, they are divided into categories, such as psychrophiles, mesophiles, thermophiles, and hyperthermophiles.[22, 23]

Many of these organisms are made up of high percentages of water, requiring a certain degree of humidity to propagate and obtain nutrients from this aqueous environment. Therefore, osmosis/osmotic pressure, oxygen, and humidity are features to be considered in industrial synthesis, especially for aerobics.[22, 23]

Besides, ultraviolet (UV) light has a relevant outcome, as the effects of surface sterilization kill most microbial species. The main one causes DNA mutations by forming pyrimidine dimers, preventing transcription.[22]

As a complement, ideal pH conditions are required for correct expansion, usually physiological pH. The lethal value of this condition depends on the species.[22] Thus, for the process of obtaining the drug to be successful, it is strictly necessary to control all the factors mentioned meticulously.

## RECOMBINANT DNA

Recombinant DNA technology involves altering an organism's genetic material to acquire improved and desired characteristics in it or its products, involving the insertion of a DNA piece with a gene sequence through an appropriate vector, often a plasmid or bacteriophage, for transport into a host cell. Depending on the procedure's purpose, the vector can help amplify and/or express the foreign DNA insert.[7, 8]

This method has generated an impact on human health. Several pharmaceutical categories are being manufactured, such as hormones, hematopoietic growth factors, blood coagulation products, thrombolytic agents, anticoagulants, interferons, interleukins, and therapeutic enzymes.[22, 23]

Since the mid-1990s, the FDA has approved many of these drugs for complex or difficult-to-treat conditions such as human immunodeficiency syndrome (AIDS), cancer, hereditary disorders, diphtheria, hepatitis, and multiple sclerosis.[7] Nevertheless, its production cost is high due to slow growth and expensive nutrient media.[22]

Prokaryotic (bacteria) or eukaryotic (yeasts) systems are common hosts for producing the desired recombinant DNA product. They offer advantages because of the high recombinant protein expression level, rapid growth, utilization of simple media, and high adaptability to large-scale production.[22]

### Techniques for recombinant DNA generation

The production of therapeutics through recombinant DNA technology has benefits. Two are the supply of drugs that could not be produced by conventional methods and the manufacture of sufficient quantities of safe, pure, and effective drugs.[22]

There are three main methods by which recombinant DNA is created: transformation, phage introduction, and non-bacterial transformation.[24] They are described below.

#### *Transformation*

In this process, a piece of DNA is inserted into a vector. Once inside, an enzymatic cleavage with restriction enzymes for target sequences' DNA sites is done. DNA fragments are obtained, which, through the action of ligases, are going to be attached to the selected vector. The organism's genome is manipulated by introducing one or more genes and new elements that regulate, decrease, or block the expression of endogenous genes. The material is introduced into a host organism and grown in culture to produce multiple copies of the inserted fragment. Finally, clones containing the relevant DNA fragment[7,8,24] are selected and collected.

The host cells must be prepared to receive the foreign DNA in the vector during this process. Distinct markers

are available to distinguish between transformed and non-transformed cells.[24]

One is an antibiotic-resistance gene. With this element, those cells that do not contain the appropriate vector die when exposed to certain antibiotics.[24]

Another way to identify colonies is white-blue or LacZ staining. This strategy involves microorganisms such as *Escherichia coli*, whose genetic material and the plasmid DNA contain portions of the lacZ gene. The union creates a functional gene that codes for the enzyme β-galactosidase, which can hydrolyze X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside), generating a blue-colored product. Such staining indicates the presence of the complete lacZ gene.[9, 25, 26, 27]

In contrast, if the plasmid incorporates the foreign DNA, the gene loses its integrity. When the plasmid is introduced, and the transformation occurs, the restriction enzymes act on the multiple cloning site (MCS), cutting the LacZ gene.[9, 25, 26, 27]

Inactivation of the LacZ gene stops β-galactosidase production, which would prevent the hydrolysis mentioned above. In this case, the colonies would be white. By these colors, it is possible to differentiate bacterial colonies with the DNA of interest from those without the desired genetic material.[9, 25, 26, 27]

***Phage display (transfection)***

This procedure is similar to transformation, except that bacteriophages or phages are involved. These are extraordinarily robust and stable viral particles, capable of infecting bacteria and replicating within them, and can be obtained in large quantities. The methodology requires *in vitro* packages and phages such as lambda or MI3 to create plates with recombinant elements.[24,28,29,30]

***Non-bacterial transformation***

This strategy has similarities to transformation, except that bacteria are not needed since the DNA is inserted directly into the nucleus of the cells through microinjections with high-velocity microprojectiles constructed of tungsten and gold. These particles have previously been coated with the DNA of interest.[24, 31, 32]

Whatever technique is selected, DNA manipulation in the host cell allows it to express the protein the inserted foreign genetic material provides. Though, certain expression factors in the host cell must be engineered. These elements comprise the promoter, the ribosome binding site, and the terminator. All these signals are in the expression vectors. The ultimate result is the synthesis of proteins to face multiple situations, such as treating specific illnesses.[8, 24, 31, 32]

## MICROORGANISMS FREQUENTLY EMPLOYED FOR BIOLOGICAL AND BIOTECHNOLOGICAL PROCESSES

On many occasions, the processes mentioned earlier involve the manipulation of microorganisms under certain conditions to obtain a specific substance. The existing list is extensive and grows because of the multiple daily studies.

**Bacteria**

They are the most efficient producers of heterologous proteins. Some reasons are the wide variety of molecular tools for their genetic manipulation, knowledge about their genomes and metabolic pathways, high cell density cultivation (HCDC) capacity and growth rate, and high yield of recombinant proteins. This last value can be up to 80 % of its dry weight.[14]

*E. coli* was explored during the first experiments. As a result of its functionality, it is still the most widely used for recombinant proteins. Its multiple advantages comprise low cost, simplicity of cultivation, and the ability to grow at high cell densities in suitable bioreactors by HCDC techniques, with the consequent fabrication of large amounts of heterologous proteins.[14] In this way, it has positioned itself as a preferred option for manufacturing therapeutic proteins on laboratory and industrial scales.

Specific strains for recombinant proteins, such as BL21 and AD494, have been acquired thanks to molecular tools. Their choice depends on the needs for recombinant molecule production (size, presence of disulfide bonds, among others). The problem is its inability to perform post-translational modifications (PTMs) and the absence of mechanisms to export proteins accumulating intracellularly.[33] Nonetheless, it allows the production of substances with a high impact on public health, such as insulin, indicated for type I and II diabetes mellitus.[4]

Another relevant bacterium is *Bacillus megaterium*. It possesses an exciting production system that lacks endotoxins associated with the outer membrane and a high secretion capacity. It has produced vitamin B12 (in the presence and absence of oxygen), penicillin amidase, and α-amylase.[34]

For its part, *Streptomyces griseus* is a microorganism of ecological and historical relevance capable of synthesizing streptomycin, a broad-spectrum aminoglycoside antibiotic that was the first to be discovered. It is considered for treating tuberculosis.[35, 36]

**Yeasts**

They have diverse applications, like producing beer, wine, enzymes, amino acids, and probiotics. They can produce proteins, enzymes, hormones, vaccines, and toxins at the pharmaceutical level.[37]

Yeasts are adapted to grow in several media, and their cultivation is practical because of the variety of substrates available for their growth. Additionally, they are capable of PTMs, unlike bacteria. These PTMs are relevant as they allow the cell to rapidly, dynamically, and reversibly modulate protein activity. Some functions that yeasts can perform are proteolytic processing, folding, disulfide bond formation, and glycosylation.[37, 38, 39]

In addition, some of these microorganisms can employ methanol as a carbon and energy source. They multiply, obtaining high cell densities in non-complex media, and this ability helps synthesize recombinant proteins.[40]

One of the organisms with this ability is *Pichia pastoris*. Some pharmaceutical products have been developed, such as interleukin 2 or IL-2 (indicated for various types of cancer),[41] human serum albumin (plasma expander),[42] and Kalbitor® (treatment of hereditary angioedema, a disease of genetic origin that affects the skin of the extremities, face, and genitals, the mucous membranes of the upper respiratory tract, and the digestive tract, characterized by episodes of swelling, without urticaria or pruritus).[40, 43]

Furthermore, thermotolerant yeasts have allowed the generation of appliances in the medical industry. These microorganisms have greater viability, metabolic activity, and fermentation rate. At the same time, they reduce contamination and cooling costs and decrease the formation of undesirable byproducts derived from cell lysis.[37]

An example is *Saccharomyces cerevisiae*. This microorganism is suitable for producing insulin and vaccines against the hepatitis virus and the human papillomavirus. Some factors that can affect protein synthesis are the isoelectric point, the formation of complexes, and the temperature.[37]

Another example is *Saccharomyces boulardii*. It is of natural origin (it has not been genetically modified), isolated from lychee fruit, and marketed as a probiotic medicine, being able to restore the gut microbiota.[44]

The production of biopharmaceuticals from yeasts has been extensively studied. Conversely, as with fungi, there are limitations related to the high purification cost of some molecules and the difficulty of their production.[4]

**Algae**

Marine sources make it possible to achieve essential medicines against pathologies such as cancer. This situation is demonstrated by the substances found in algae, which have shown clinical and therapeutic effects.[45] For example, isolating two nucleosides with arabinose from the sponge *Cryptotethya crypta* allowed cytarabine production, which was the first drug of marine origin approved.[16] It is applied alone or with other chemotherapy drugs to treat certain types of leukemia, including acute myeloid leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, and meningeal leukemia.[46]

Microalgae have unique characteristics such as photosynthetic capacity and diversity of bioproducts. They are of great interest for their role as cell factories for the generation of drugs. Their pigments have shown toxicity absence and biological activity to prevent acute and chronic coronary syndromes, atherosclerosis, rheumatoid arthritis, muscular dystrophy, and neurological disorders.[17]

These microorganisms generate compounds to adapt and survive in numerous environmental conditions. Many species produce a high percentage of total lipids (30 to 70 % of dry weight). The accumulation of fatty acids is linked to growth stages, functioning as an energy reserve for cell division, even under unfavorable conditions.[17]

Among the notable species is *Chlamydomonas reinhardtii*. It has a high growth rate, a stable genetic transformation that allows large-scale production of recombinant proteins, ease of cultivation in bioreactors, and no evidence of toxic or mutagenic components at the cellular level. It has been considered to manufacture vaccines against malaria and human papillomavirus tumors and hormones.[47]

On the other hand, the microalga *Dunaliella salina* has been essential for vaccines (e.g., influenza). It is accessible to culture and to manipulate and performs PTMs. The microorganism is halotolerant (reducing the risk of contamination with other mechanisms) and lacks a rigid cell wall, facilitating the introduction of genes into the cell.[48]

Based on the above, **Table 1** shows the main advantages and disadvantages of the mentioned microorganisms for generating proteins.

**Table 1: Advantages and disadvantages of microorganisms for protein production.[4, 14, 33, 37, 38, 48]**

| Microorganisms | Advantages | Disadvantages |
|---|---|---|
| **Bacteria** | -Well-developed molecular tools.<br>-Extensive knowledge about their genomes and metabolic pathways.<br>-High growth rate.<br>-High yield for the synthesis of recombinant proteins. | -Cannot make some of the PTMs required by many proteins to be fully active. |
| **Yeasts** | -Can do PTMs.<br>-Adapted to grow on solid media.<br>-Practical cultivation. | -High cost for the purification of some molecules.<br>-Difficult production. |
| **Algae** | -Can achieve PTMs.<br>-Low cost.<br>-Easy cultivation.<br>-Require less time for the transformation process.<br>-Stable transgenic lines are available. | -Some require modifications to obtain a weakened cell wall or undergo enzymatic degradation of this structure. |

## BIOLOGICAL AND BIOTECHNOLOGICAL DRUGS OBTAINED THROUGH MICROORGANISMS

There are distinct medicines obtained from biological sources or by biotechnological techniques. This fact demonstrates the importance and potential of microorganisms as promising sources of human therapies against different illnesses.

### Antineoplastics

In 2018, 18.1 million cases and 9.6 million cancer-related deaths were reported worldwide. Given this panorama, drugs against this pathology have become an urgent need. The search for new strategies and molecules is extremely necessary.[18]

#### *Marizomib (saninosporamide A)*

It is the most advanced secondary metabolite of marine actinomycetes in drug development. The compound was isolated from *Salinispora tropica* in 2003.[49] Marizomib is a promising drug that shows better clinical activity in cancers resistant to bortezomib.[50]

It belongs to the β-lactones, and its mechanism of action consists of the proteasome irreversible inhibition. The molecule is orally bioactive and inhibits all three proteasome proteolytic activities. The investigations are centered on treating multiple myeloma and glioblastoma through phase III clinical studies.[16, 45, 51]

A phase I/II clinical study in patients with recurrent glioblastoma was conducted in three stages:[52]

- Stage 1: marizomib was administered as a 10-minute intravenous infusion once a week for three weeks in 28-day cycles at a dose of 0.8 mg/m$^2$. Dose and infusion time was the recommended phase II dose established on earlier investigations in solid tumors and multiple myeloma.
- Stage 2: a phase I study was done, with dose escalation 3 plus 3 in combination with the monoclonal antibody bevacizumab, followed by a phase II expansion cohort. Marizomib was administered as a 10-minute intravenous infusion in dose cohorts ranging from 0.55 to 0.8 mg/m$^2$ on days 1, 8, and 15 of each 28-day cycle. Bevacizumab was administered intravenously at a fixed dose of 10 mg/kg on days 1 and 15 of each 28-day cycle in all patients. An expansion cohort at the recommended phase II dose was incorporated to assess the safety and efficacy of the combination therapy.
- Stage 3: in this phase, the activity of marizomib and bevacizumab using intrapatient dose escalation of marizomib based on specific central nervous system adverse effects in a patient population similar to stage 2 was assessed.

From all the above, it was determined that its safety profile as monotherapy and in combination was consistent with previous observations regarding its ability to cross the blood-brain barrier. Nonetheless, preliminary efficacy did not demonstrate a significant benefit of its addition to bevacizumab against recurrent glioblastoma.[52]

#### *Actinomycin D (dactinomycin)*

It is a leading member of the actinomycins, a family of structurally related chromo peptide antibiotics with a common phenoxazine chromophore linked to a pair of pentapeptide lactone moieties. These antibiotics vary in amino acid content. Actinomycin is one of the oldest compounds in this pharmacological group and the first to show activity against cancer.[53] It has been approved as a therapeutic option for people with malignancies such as localized recurrent solid tumors, Wilms tumor, choriocarcinoma, testicular cancer, Ewing sarcoma, and rhabdomyosarcoma, either as monotherapy or in combination with other medications.[54]

This substance is produced by *Streptomyces parvulus* and *Streptomyces antibioticus*.[54] It is a hybrid compound since it acts as a DNA intercalator and a

binding agent to the minor groove of this genetic material.[55]

In a phase III non-inferiority clinical study conducted with 57 participants, the treatment regimen was randomly assigned, 28 persons received actinomycin D, and 26 patients were administered methotrexate. In the first case, a regimen of 1.25 mg/m$^2$ intravenously was given every 14 days (maximum 2 mg). In contrast, for the second group, 0.4 mg/kg intravenously was given for 5 days every 14 days (maximum 25 mg) or 50 mg intramuscularly on days 1, 3, 5, and 7 (four doses per cycle) in combination with leucovorin 15 mg orally on days 2, 4, 6, and 8, 24 to 30 hours prior to injection. This latter regimen was repeated every 14 days. The main results showed more toxicity with methotrexate and no significant difference in patients' quality of life, body image, sexual function, and treatment side effects. Likewise, good tolerance to both was observed since no patient had to discontinue them.[56]

In another case, a phase III trial compared regimens of weekly methotrexate with pulsed dactinomycin for low-risk gestational trophoblastic neoplasia. Of the 216 eligible patients, 107 received biweekly intravenous dactinomycin 1.25 mg/m$^2$ and 109 weekly intramuscular methotrexate 30 mg/m$^2$. The most significant finding was that the antibiotic regimen exhibited a higher complete response rate than methotrexate for this illness.[57]

***Romidepsin***

Initially, it was isolated from *Chromobacterium violaceum* in Japan, and its antitumor activity was later found *in vitro* and *in vivo*. Romidepsin (FK228 or FR901228) is a small molecule belonging to the bicyclic peptide selective inhibitors of class I histone deacetylases (HDACs).[54, 58, 59]

HDACs are involved in regulating histones, vital components of the nucleosome structure. Histone acetyltransferases, like deacetylases, act dynamically to regulate DNA transcription and subsequent protein expression. These enzymes are often overexpressed in malignant cells, driving malignant transformation.[59]

The activity of romidepsin as a novel HDAC inhibitor was first described by a Japanese group looking for drugs to induce transcriptional activation. Initially, its mechanism of action was unknown, but the ability to induce selective apoptosis of malignant cells was observed.[59] The disulfide bond of the prodrug is reduced within the cell, generating a thiol functional moiety that reversibly interacts with the zinc atom in the Zn-dependent histone deacetylase binding pocket. Such interaction inhibits the biological activity of the enzyme and can restore normal gene expression in cancer cells, with consequent cell cycle arrest and apoptosis.[58]

The FDA approved it for relapsed T-lymphocytic carcinoma in 2009 and peripheral T-lymphocytic carcinoma in 2011. Its cytotoxicity is not limited to hematologic disorders since the possibility of testing it against human immunodeficiency virus (HIV) has even been appreciated.[58, 60, 61]

In a phase I/II clinical study conducted with 25 patients, the objective was to evaluate the study regimens' clinical activity and adverse events. The combination of bortezomib and dexamethasone with romidepsin was well tolerated, and it demonstrated substantial efficacy in a group of heavily pretreated patients. All patients received bortezomib 1.3 mg/m$^2$ on days 1, 4, 8, and 11 and dexamethasone 20 mg on days 1, 2, 4, 5, 8, 9, 11, and 12. Romidepsin was administered to 10 patients, starting with 8 mg/m$^2$ intravenously on days 1, 8, and 15 every 28 days, with a planned, accelerated intrapatient dose increase to 10, 12, and 14 mg/m$^2$.[62]

**Antibiotics**

The rise and spread of antibiotic resistance represent a unique challenge, as it causes traditional regimens to fail, resulting in an increase in morbidity and mortality associated with pathogenic bacteria.[63] It is estimated that approximately 700,000 people die each year from this situation.[64]

Therefore, there is a challenge in combating bacterial infections. Due to the few effective therapeutic options, the lack of successful prevention measures, and the investigation of insufficient new molecules, the challenge is finding new strategies for developing alternative antimicrobial treatments.[63] Some of these agents likely to be commercialized are listed below.

***2-allyloxyphenol***

It is obtained from the actinobacterium *Streptomyces* sp.[49, 65, 66] It has been found to inhibit 21 bacteria (both Gram-positive and Gram-negative) and three fungi in the trough range of 0.2 to 1.75 mg/ml, determined by the agar diffusion method. The required concentration was higher for fungi. At the same time, its antioxidant capacity was demonstrated, with a half maximal inhibitory concentration ($IC_{50}$) value equal to 22 μg ml$^{-1}$, measured by 1,1-diphenyl-2-picryl hydrazyl (DPPH) scavenging activity.[66]

The inhibitory activity of p-alkoxyphenols has been explained through molecular docking studies in *E. coli* and mice. These molecules interrupt the catalytic electron transfer pathway between protein R1 and the tyrosyl radical in protein R2 of the ribonucleotide reductase enzyme, forming hydrogen bonds with tryptophan 48 (Trp48) and aspartic acid 237 (Asp237).[67]

***Essramicyn***

It is the first 1,2,4-triazolo[1,5-a] pyrimidine-type antibiotic.[68] The compound was isolated from the culture broth of *Streptomyces* sp. marine species found in

the Mediterranean Sea's sediment.[49, 65, 68, 69] It has shown considerable antimicrobial activity against Gram-positive and Gram-negative microorganisms (minimum inhibitory concentration or MIC between 1.0 and 2.0 to 8.0 µg/ml) from bacterial strains such as *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Micrococcus luteus*[68, 69, 70] and an inhibition rate greater than 50 % against 14 types of phytopathogenic fungi at 50 µg/ml.[68] Its mechanism of action is not fully elucidated.[71]

In greater detail, triazolopyrimidines are smooth muscle cell growth inhibitors with the potential to relieve and prevent cardiovascular pathologies. Before the isolation of essramycin, synthetic production was the only strategy to obtain these molecules.[72]

***Anthracimycin***

This compound is obtained from a bacterial isolate of *Streptomyces* sp. in marine sediments found near Santa Barbara in California, United States.[19] It is an unusual 14-membered macrolide produced by a trans-acyltransferase PKS system in two marine streptomycetes, *Streptomyces* sp. CNH365 and *Streptomyces* sp. T676. This molecule has been investigated using heterologous hosts, specifically *S. coelicolor*, and gene pool transfer to express partial or complete biosynthetic pathways.[73]

This substance has potent antibacterial activity against the anthrax-causing agent *Bacillus anthracis* (strain UM23C1–1), with a MIC of 0.079 µM. As a complement, some biological studies demonstrated its antistaphylococcal activity *in vitro* and *in vivo* against a broad panel of *S. aureus* strains, including meticillin-sensitive *S. aureus* (MSSA), meticillin-resistant *S. aureus* (MRSA), and vancomycin-resistant *S. aureus*, with MIC values of 0.63 µM or greater.[74]

***Pargamicin A***

This compound has been isolated from the culture of *Amycolatopsis* sp. strain ML1-hF4.[75, 76] Its structure is a cyclic peptide containing N-methyl-3-hydroxy valine, 4-hydroxy piperazic acid, sarcosine, phenylalanine, N-hydroxy isoleucine, and piperazic acid. It shows excellent antibacterial activity *in vitro*, comparable to or more potent than commercially available drugs, including vancomycin.[77]

Among the studies found, the molecule showed potent antibacterial activity against strains of *S. aureus*, such as MRSA, and vancomycin-resistant *Enterococcus faecalis*/*faecium* (VRE).[75, 78] Its mechanism of action involves the depolarization of the membrane and the loss of its function, affecting the integrity of this cellular structure.[76, 77]

Other antibiotics obtained from microorganisms are already on the market for numerous infections. Two of them are tetracycline and streptomycin.

***Tetracycline***

It is a broad-spectrum polyketide antibiotic produced by bacteria of the genus *Streptomyces*.[79] Tetracyclines were discovered in the 1940s and exhibited activity against many microorganisms, including Gram-positive and Gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae, and protozoan parasites. These low-cost antibiotics are widely dispensed for human and animal infections because of their undesired effects profile. Moreover, it is administered at subtherapeutic levels for animal feed as a growth promoter.[80]

This molecule penetrates bacterial cells by passive diffusion and inhibits bacterial growth by interfering with protein synthesis or destroying the membrane.[81] Specifically, it binds to the 30S ribosomal subunit, interfering with aminoacyl-tRNA (from transfer ribonucleic acid) binding to the mRNA-ribosome complex, affecting the production of pathogen proteins.[82]

Regarding the investigations, in a randomized, multicenter, double-blind, placebo-controlled study carried out with 284 patients, three doses (0.75, 1.5, or 3.0 mg/kg/day for 12 weeks) administered orally in the form of capsules were compared to placebo for moderate to severe facial acne vulgaris treatment. The two higher concentrations were shown to have greater efficacy than the placebo.[83]

***Streptomycin***

It was the first aminoglycoside antibiotic elucidated, initially isolated from *Streptomyces griseus*. As with other aminoglycosides, it is bactericidal. The molecule interferes with ribosomal peptide/protein synthesis by binding to a side 16S rRNA located on the smaller 30S component of the bacterial ribosome. Such interaction affects its functionality and stops protein synthesis by preventing the formation of peptide bonds.[84]

Its main indication is for multidrug treatment of pulmonary tuberculosis. In addition, it has activity against several Gram-negative aerobic bacteria.[84]

Therefore, it has been studied with other antibiotics for different infections. A clinical study evaluated the efficacy of a combination of rifampin and streptomycin against *Mycobacterium ulcerans* infection. It was made with 160 patients, administering oral rifampin at 10 mg/kg and intramuscular streptomycin at 15 mg/kg for eight weeks. In 152 patients with all forms of disease, from early nodules to large ulcers, with or without edema, the lesions healed without surgery. Besides, its safety was demonstrated since adverse reactions were rare.[85]

**Inmunosupresors**

Two of the most potent immunosuppressants known to date are cyclosporine A and FK506, and both have revolutionized organ transplantation. Likewise, they are

effective neuroprotectants, so they have appreciably impacted neurology.[86]

Immunosuppressants are commonly administered for treating autoimmune illnesses, such as rheumatoid arthritis and lupus, and for organ transplantation. It is estimated that approximately 6 million Americans are taking these medications to weaken their immune system. Their study has even been associated with SARS-CoV-2 for improving the response of the antibodies generated by the vaccine against this virus through studies in animal models.[87]

***Cyclosporin A***

Cyclosporin A was detected while searching for new antifungal agents. It was found to have immunosuppressive properties, transforming procedures associated with organ transplantation. Furthermore, indications have been approved for psoriasis, rheumatoid arthritis, and uveitis.[20] Fermentation techniques with *Tolypocladium inflatum* produce the molecule.[88]

Its immunosuppressive effects can be attributed to a ternary complex between the compound cyclophilin A and the two subunits of the phosphatase calcineurin. This complex decreases the production of proinflammatory cytokines in T lymphocytes.[20]

In a randomized, open clinical study, its efficacy was evaluated in combination with interferon-α (IFN-α) versus the administration of the cytokine alone in 120 patients with chronic hepatitis C. Patients received IFN-α alone (44 patients) or the drug combination for 24 weeks, with follow-up for another 24 weeks. The virologic response at 12 and 48 weeks was shown to be significantly higher in patients treated with combination therapy. As an adjunct, more patients showed normalization of blood alanine aminotransferase (ALT) at 48 weeks, although there was no difference during or at the end of treatment.[89]

***Rapamycin***

Rapamycin was discovered to be an antifungal metabolite produced by *Streptomyces hygroscopicus*. The soil sample was obtained on Easter Island or Rapa Nui.[90] Another name for the drug is sirolimus.[91]

It is a potent inhibitor of ribosomal protein S6 kinase beta-1 (S6K1) activation, a serine/threonine kinase activated by various agonists, and a relevant mediator of phosphoinositide 3-kinase (PI3K) signaling. At the same time, the target of rapamycin (TOR) was identified in yeast and animal cells.[90]

Its mechanism of action involves forming a gain-of-function complex with the 12 kDa FK506-binding protein (FKBP12). The scaffold binds to and participates as an allosteric inhibitor of mammalian TOR complex 1 (mTORC1).[90]

Within the investigations, the response was evidenced by a multicenter systematic retrospective review of patients treated with oral sirolimus and a prospective phase II clinical trial assessing its efficacy and safety in complicated vascular anomalies. The doses given were:[92]

- ✓ Prospective study: a liquid formulation was given at a dose of 0.8 mg/m$^2$ twice daily, with adjustments for a goal trough level of 10 to 15 ng/ml.
- ✓ Retrospective study: an oral form was administered, either liquid or tablet, one to two times a day with adjustments for individualized goal trough levels, always considering a range of 8 to 15 ng/ml.

The findings indicated that this drug effectively stabilizes or reduces signs and symptoms in patients with generalized lymphatic anomaly and Gorham-Stout disease. As a complement, it increased the quality of life in these individuals.[92]

**Hormones: human recombinant insulin**

In 1955, there were 135 million diabetic patients. Forecasts estimate around 300 million by 2025. In addition, between 1995 and 2025, an increase of 35 % in prevalence has been estimated, with a predominance in females and the age group of 45 to 64 years.[93]

The drug administered for this condition is insulin. The recombinant protein is produced through a gene incorporated into a plasmid and inserted in *E. coli*, specifically the K-12 strain. The extracted material is processed and purified in several steps by suitable extraction chromatography.[94]

This recombinant molecule is identical to endogenous human insulin, except for the transposition of two amino acids (from proline-lysine to lysine-proline at positions 28 and 29 on the B chain).[95] Recombinant human insulin's purity and pharmaceutical quality are superior to animal and semi-synthetic insulin. Its clinical efficacy is well-defined over 35 years of utilization.[96, 97]

Finally, **Table 2** summarizes the drugs commercialized and under investigation mentioned above. Moreover, their pharmacological group and the microorganism or microorganisms from which they were obtained are established.

**Table 2: Antineoplastics, antibiotics, immunosuppressors, and hormones obtained from microorganisms.**

| Pharmacological group | Drug | Natural source |
|---|---|---|
| **Antineoplastics** | Marizomib[49] | *S. tropica* |
| | Actinomycin D[54] | *S. parvenus*<br>*S. antibioticus* |
| | Romidepsin[54, 58, 59] | *C. violaceum* |
| **Antibiotics** | 2-allyloxyphenol[49, 65, 66] | *Streptomyces* sp. |
| | Essramycin[49, 65, 68, 69] | *Streptomyces* sp. |
| | Anthracimycin[19] | *Streptomyces* sp. |
| | Pargamicin A[75, 76] | *Amycolatopsis* sp. |
| | Tetracycline[79] | *Streptomyces* sp. |
| | Streptomycin[84] | *S. griseus* |
| **Immunosuppressors** | Cyclosporin A[88] | *T. inflatum* |
| | Rapamycin[90] | *S. hygroscopicus* |
| **Hormones** | Recombinant human insulin[94] | *E. coli* |

## CONCLUSIONS

Microorganisms have been employed since ancient times to make beer, wine, and cheese. Their use, together with the discovery of the structure of DNA, has changed a wide variety of study fields, highlighting everything related to the prevention and treatment of diseases.

Thanks to them and the genetic modifications achieved through recombinant DNA technology, bacteria, yeasts, and algae are living factories that can obtain biological and biotechnological drugs for pathologies with reserved or difficult prognoses. Some therapeutic groups produced this way embrace antineoplastics, antibiotics, immunosuppressants, and hormones.

Despite the success achieved, the findings continue, which is reflected in the multiple efforts to gain an in-depth knowledge of the resources offered by nature to obtain substances with varied pharmacological effects. In this way, it is expected that in the short term, more microorganisms will be available to synthesize compounds that are difficult to obtain or are in a specific research phase.

## REFERENCES

1. Biotechnology, American Chemistry Society, https://www.acs.org/careers/chemical-sciences/fields/biotechnology.html.
2. Oosting RS. Molecular Biotechnology: From DNA Sequence to Therapeutic Protein. 5th ed. In: Crommelin DJA, Sindelar RD, and Meibohm B (eds.). Pharmaceutical Biotechnology: Fundamentals and Applications, Cham; Springer Nature Switzerland AG: 2019.
3. Rodriguez Cumplido D, Asensio Ostos C. Fármacos biológicos y biosimilares: aclarando conceptos. Aten Primaria, 2018; 50(6): 323-4.
4. Jozala AF, Geraldes DC, Tundisi LL, Feitosa VA, Breyer CA, Cardoso SL, et al. Biopharmaceuticals from microorganisms: from production to purification. Braz J Microbiol, 2016; 47(Suppl 1): 51-63.
5. Jagschies G. Selected Biotherapeutics Overview. In: Jagschies G, Lindskog E, Łącki K, and Galliher P (eds.). Biopharmaceutical Processing: Development, Design, and Implementation of Manufacturing Processes, Massachusetts; Elsevier: 2018.
6. Lai YH, Ambsberry KL. Specification of biotechnology products. 2nd ed. In: Riley CM, Rosanke TW, and Reid G (eds.). Specification of Drug Substances and Products: Development and Validation of Analytical Methods, Massachusetts; Elsevier: 2020.
7. Ijaz S, Ul Haq I. Recombinant DNA Technology. Newcastle upon Tyne; Cambridge Scholars Publishing: 2019.
8. Khan S, Ullah MW, Siddique R, Nabi G, Manan S, Yousaf M, et al. Role of Recombinant DNA Technology to Improve Life. Int J Genomics, 2016; 2016: 2405954.
9. Stryjewska A, Kiepura K, Librowski T, Lochyński S. Biotechnology and genetic engineering in the new drug development. Part I. DNA technology and recombinant proteins. Pharmacol Rep, 2013; 65(5): 1075-85.
10. Alvarado-Madrigal MF, Chavarría-Quirós T, Leiva-Montero B, Mora-Román JJ. Producción de proteínas recombinantes a partir de animales transgénicos: Sistemas y aplicaciones. Tecnología en Marcha, 2019; 32(4): 133-44.
11. Vector, National Human Genome Research Institute, https://www.genome.gov/genetics-glossary/Vector.
12. Buchholz K, Collins J. The roots--a short history of industrial microbiology and biotechnology. Appl Microbiol Biotechnol, 2013; 97(9): 3747-62.
13. Devi S. Microbes as Natural Products for Drug Discovery. In: Patra JK, Shukla AC, and Das G (eds.). Advances in Pharmaceutical Biotechnology: Recent Progress and Future Applications, Singapore; Springer Nature Singapore: 2020.
14. Matejczyk M. Environmental and Recombinant Microorganisms for Biopharmaceuticals Production. Civil and Environmental Engineering, 2014; 5(1): 15-21.

15. Bertrand S, Bohni N, Schnee S, Schumpp O, Gindro K, Wolfender JL. Metabolite induction via microorganism co-culture: a potential way to enhance chemical diversity for drug discovery. Biotechnol Adv, 2014; 32(6): 1180-204.
16. Pereira RB, Evdokimov NM, Lefranc F, Valentão P, Kornienko A, Pereira DM, et al. Marine-Derived Anticancer Agents: Clinical Benefits, Innovative Mechanisms, and New Targets. Mar Drugs, 2019; 17(6): 329.
17. Ramana KV, Xavier JR, Sharma RK. Recent Trends in Pharmaceutical Biotechnology. Pharm Biotechnol Curr Res, 2017; 1(1): 5.
18. Ding Z, Ma M, Zhong C, Wang S, Fu Z, Hou Y, et al. Development of novel phenoxy-diketopiperazine-type plinabulin derivatives as potent antimicrotubule agents based on the co-crystal structure. Bioorg Med Chem, 2020; 28(1): 115186.
19. Barbosa F, Pinto E, Kijjoa A, Pinto M, Sousa E. Targeting antimicrobial drug resistance with marine natural products. Int J Antimicrob Agents, 2020; 56(1): 106005.
20. Fu J, Becker C, Cao L, Capparelli M, Denay R, Fujimoto R, et al. Development of a cyclosporin A derivative with excellent anti-hepatitis C virus potency. Bioorg Med Chem, 2018; 26(4): 957-69.
21. Donova MV, Egorova OV. Microbial steroid transformations: current state and prospects. Appl Microbiol Biotechnol, 2012; 94(6): 1423-47.
22. Bisen PS, Debnath M, Prasad GBKS. Microbes: Concepts and Applications. New Jersey; Wiley Blackwell: 2012.
23. Singhania RR, Patel AK, Thomas L, Pandey A. Solid-State Fermentation. In: Wittman C and Liao JC (eds.). Industrial Biotechnology: Products and Processes, Weinheim; Wiley-VCH: 2017.
24. Taylor S. The Past, Present, and Future of Recombinant DNA Technology. Science Department Journal, 2019; 1(1): 23-40.
25. Juers DH, Matthews BW, Huber RE. LacZ β-galactosidase: Structure and function of an enzyme of historical and molecular biological importance. Protein Sci, 2012; 21(12): 1792-807.
26. Burn SF. Detection of β-Galactosidase Activity: X-gal Staining. In: Michos O (ed.). Kidney Development: Methods and Protocols, New York; Humana Press: 2012.
27. Trifonov S, Yamashita Y, Kase M, Maruyama M, Sugimoto T. Overview and assessment of the histochemical methods and reagents for the detection of β-galactosidase activity in transgenic animals. Anat Sci Int, 2016; 91(1): 56-67.
28. Smith GP. Phage Display: Simple Evolution in a Petri Dish (Nobel Lecture). Angew Chem Int Ed Engl, 2019; 58(41): 14428-37.
29. Petrenko VA. Landscape Phage: Evolution from Phage Display to Nanobiotechnology. Viruses, 2018; 10(6): 311.
30. Barderas R, Benito-Peña E. The 2018 Nobel Prize in Chemistry: phage display of peptides and antibodies. Anal Bioanal Chem, 2019; 411(12): 2475-9.
31. Akram M, Jabeen F, Daniyal M, Zainab R, ul Haq U, Adetunji CO, et al. Genetic Engineering of Novel Products of Health Significance: Recombinant DNA Technology. In: Egbuna C and Dable-Tupas G (eds.). Functional Foods and Nutraceuticals Bioactive Components, Formulations and Innovations, Cham; Springer Nature Switzerland AG: 2020.
32. Jember TF. Nanobody cDNA Mock-Up in pHEN6c Plasmid Vector: Live Out. Am J Mol Biol, 2021; 11(4): 116-28.
33. Lara AR. Producción de Proteínas Recombinantes en *Escherichia coli*. Rev Med Ing Quim, 2011; 10(2): 209-23.
34. Tórtolo-Cabañas K, Bell-García A. Producción de proteínas recombinantes en *Bacillus megaterium*: estado del arte. ICIDCA. Sobre los Derivados de la Caña de Azúcar, 2015; 49(1): 22-6.
35. Ruiz P, Rodríguez-Cano F, Zerolo FJ, Casal M. La estreptomicina como fármaco de segunda línea en la quimioterapia de la tuberculosis. Rev Esp Quimioterap, 2003; 16(2): 188-94.
36. Westhoff S, Otto SB, Swinkels A, Bode B, van Wezel GP, Rozen DE. Spatial structure increases the benefits of antibiotic production in *Streptomyces*. Evolution, 2020; 74(1): 179-87.
37. Mejía-Barajas JA, Montoya-Pérez R, Cortés-Rojo C, Saavedra-Molina A. Levaduras Termotolerantes: Aplicaciones Industriales, Estrés Oxidativo y Respuesta Antioxidante. Inf Tecnol, 2016; 27(4): 3-16.
38. Ostos Ortíz OL, Rosas Arango SM, González Devia JL. Aplicaciones biotecnológicas de los microorganismos. Nova, 2019; 17(31): 129-63.
39. Separovich RJ, Wong MWM, Chapman TR, Slavich E, Hamey JJ, Wilkins MR. Post-translational modification analysis of *Saccharomyces cerevisiae* histone methylation enzymes reveals phosphorylation sites of regulatory potential. J Biol Chem, 2021; 296: 100192.
40. García-Suárez J, Zumalacárregui-de-Cárdenas L, Santana-Vázquez Z. Usos de la levadura *Pichia pastoris* en la producción de proteínas recombinantes. Vaccimonitor, 2021; 30(3): 153-63.
41. Atkins MB. Interleukin-2: Clinical Applications. Semin Oncol, 2002; 29(3 Suppl 7): 12-7.
42. Caraceni P, Tufoni M, Bonavita ME. Clinical use of albumin. Blood Transfus, 2013; 11(Suppl 4): s18-s25.
43. Vives Toledo R, Sorlí Guerola JV, Sierra Santos L, García Ribes M. Angioedema hereditario. Rev Clin Med Fam, 2015; 8(1): 62-5.
44. Buts JP. Ejemplo de un medicamento probiótico: Saccharomyces boulardii liofilizada. Rev Gastroenterol, 2005; 25(2): 176-88.
45. Saeed AFUH, Su J, Ouyang S. Marine-derived drugs: Recent advances in cancer therapy and

immune signaling. Biomed Pharmacother, 2021; 134: 111091.
46. Cytarabine, National Library of Medicine, https://medlineplus.gov/druginfo/meds/a682222.html.
47. Carreño-Campos C, Villarreal ML, Caltempa AO. El potencial del genoma del cloroplasto de *Chlamydomonas reinhardtii* para la producción de proteínas recombinantes. BioTecnología, 2021; 25(5): 108-26.
48. Mendoza Damián C. Construcción de un Vector para Expresar la Proteína Hemaglutinina del Virus de la Influenza A (H1N1) en el Cloroplasto de *Dunaliella salina* [master thesis]. Baja California; Universidad Autónoma de Baja California: 2012.
49. Zotchev SB. Marine actinomycetes as an emerging resource for the drug development pipelines. J Biotechnol, 2012; 158(4): 168-75.
50. Seyed MA, Ayesha S. Marine-derived pipeline anticancer natural products: a review of their pharmacotherapeutic potential and molecular mechanisms. Futur J Pharm Sci, 2021; 7: 203.
51. Niewerth D, Jansen G, Riethoff LFV, van Meerloo J, Kale AJ, Moore BS, et al. Antileukemic Activity and Mechanism of Drug Resistance to the Marine *Salinispora tropica* Proteasome Inhibitor Salinosporamide A (Marizomib). Mol Pharmacol, 2014; 86(1): 12-9.
52. Bota DA, Mason W, Kesari S, Magge R, Winograd B, Elias I, et al. Marizomib alone or in combination with bevacizumab in patients with recurrent glioblastoma: Phase I/II clinical trial data. Neurooncol Adv, 2021; 3(1): vdab142.
53. Vardanyan R, Hruby V. Synthesis of Best-Seller Drugs. San Diego; Academic Press: 2016.
54. Mohan CD, Rangappa S, Nayak SC, Jadimurthy R, Wang L, Sethi G, et al. Bacteria as a treasure house of secondary metabolites with anticancer potential. Semin Cancer Biol, 2022; 86(Pt 2): 998-1013.
55. Avendaño C, Menéndez JC. Medicinal Chemistry of Anticancer Drugs. 2nd ed., Massachusetts; Elsevier: 2015.
56. Schink JC, Filiaci V, Huang HQ, Tidy J, Winter M, Carter J, et al. An international randomized phase III trial of pulse actinomycin-D versus multi-day methotrexate for the treatment of low risk gestational trophoblastic neoplasia; NRG/GOG 275. Gynecol Oncol, 2020; 158(2): 354-60.
57. Osborne RJ, Filiaci V, Schink JC, Mannel RS, Alvarez Secord A, Kelley JL, et al. Phase III trial of weekly methotrexate or pulsed dactinomycin for low-risk gestational trophoblastic neoplasia: a gynecologic oncology group study. J Clin Oncol, 2011; 29(7): 825-31.
58. Valdez BC, Brammer JE, Li Y, Murray D, Liu Y, Hosing C, et al. Romidepsin targets multiple survival signaling pathways in malignant T cells. Blood Cancer J, 2015; 5(10): e357.
59. Bertino EM, Otterson GA. Romidepsin: a novel histone deacetylase inhibitor for cancer. Expert Opin Investig Drugs, 2011; 20(8): 1151-8.
60. Xiong L, Chen CF, Min TL, Hu HF. Romipeptides A and B, two new romidepsin derivatives isolated from *Chromobacterium violaceum* No.968 and their antitumor activities *in vitro*. Chin J Nat Med, 2019; 17(2): 155-60.
61. Kitagaki J, Shi G, Miyauchi S, Murakami S, Yang Y. Cyclic depsipeptides as potential cancer therapeutics. Anticancer Drugs, 2015; 26(3): 259-71.
62. Harrison SJ, Quach H, Yuen K, Strayer A, Copeman MC, Peinert S, et al. High Response Rates with the Combination of Bortezomib, Dexamethasone and the Pan-Histone Deacetylase Inhibitor Romidepsin in Patients with Relapsed or Refractory Multiple Myeloma in a Phase I/II Clinical Trial. Blood, 2008; 112(11): 3698.
63. Frieri M, Kumar K, Boutin A. Antibiotic resistance. J Infect Public Health, 2017; 10(4): 369-78.
64. The Review on Antimicrobial Resistance. Review on Antimicrobial Resistance: Tackling Drug-Resistant Infections Globally. London; The Review on Antimicrobial Resistance: 2016.
65. Manivasagan P, Venkatesan J, Sivakumar K, Kim SK. Pharmaceutically active secondary metabolites of marine actinobacteria. Microbiol Res, 2014; 169(4): 262-78.
66. Arumugam M, Mitra A, Jaisankar P, Dasgupta S, Sen T, Gachhui R, et al. Isolation of an unusual metabolite 2-allyloxyphenol from a marine actinobacterium, its biological activities and applications. Appl Microbiol Biotechnol, 2010; 86(1): 109-17.
67. Luo J, Gräslund A. Ribonucleotide reductase inhibition by *p*-alkoxyphenols studied by molecular docking and molecular dynamics simulations. Arch Biochem Biophys, 2011; 516(1): 29-34.
68. Amin NH, El-Saadi MT, Ibrahim AA, Abdel-Rahman HM. Design, synthesis and mechanistic study of new 1,2,4-triazole derivatives as antimicrobial agents. Bioorg Chem, 2021; 111: 104841.
69. El-Gendy MMA, Shaaban M, Shaaban KA, El-Bondkly AM, Laatsch H. Essramycin: A First Triazolopyrimidine Antibiotic Isolated from Nature. J Antibiot, 2008; 61(3): 149-57.
70. Pinheiro S, Pinheiro EMC, Muri EMG, Pessôa JC, Cadorini MA, Greco SJ. Biological activities of [1,2,4]triazolo[1,5-a]pyrimidines and analogs. Med Chem Res, 2020; 29: 1751-76.
71. Mayer AMS, Rodríguez AD, Berlinck RGS, Fusetani N. Marine pharmacology in 2007-8: Marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanisms of action. Comp Biochem Physiol C Toxicol Pharmacol, 2011; 153(2): 191-222.

72. Hamedi J, Mohammadipanah F, Panahi HMS. Biotechnological Exploitation of Actinobacterial Members. In: Maheshwari DK and Saraf M (eds.). Halophiles: Biodiversity and Sustainable Exploitation, Cham; Springer Nature Switzerland AG: 2015.
73. Nepal KK, Wang G. Streptomycetes: Surrogate Hosts for the Genetic Manipulation of Biosynthetic Gene Clusters and Production of Natural Products. Biotechnol Adv, 2019; 37(1): 1-20.
74. Liu M, El-Hossary EM, Oelschlaeger TA, Donia MS, Quinn RJ, Abdelmohsen UR. Potential of marine natural products against drug-resistant bacterial infections. Lancet Inf Dis, 2019; 19(7): e237-e245.
75. Igarashi M, Sawa R, Kinoshita N, Hashizume H, Nakagawa N, Homma Y, et al. Pargamicin A, a novel cyclic peptide antibiotic from *Amycolatopsis* sp. J Antibiot, 2008; 61(6): 387-93.
76. Hashizume H, Adachi H, Igarashi M, Nishimura Y, Akamatsu Y. Biological activities of pargamicin A, a novel cyclic peptide antibiotic from *Amycolatopsis* sp. J Antibiot, 2010; 63(6): 279-83.
77. Hashizume H, Sawa R, Yamashita K, Nishimura Y, Igarashi M. Structure and antibacterial activities of new cyclic peptide antibiotics, pargamicins B, C and D, from *Amycolatopsis* sp. ML1-hF4. J Antibiot, 2017; 70(5): 699-704.
78. Abbas M, Ovais M, Atiq A, Ansari TM, Xing R, Sprujit E, et al. Tailoring supramolecular short peptide nanomaterials for antibacterial applications. Coord Chem Rev, 2022; 460: 214481.
79. Tetracycline, Drugbank Online, https://go.drugbank.com/drugs/DB00759.
80. Chopra I, Roberts M. Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. Microbiol Mol Biol Rev, 2001; 65(2): 232-60.
81. Schnappinger D, Hillen W. Tetracyclines: antibiotic action, uptake, and resistance mechanisms. Arch Microbiol, 1996; 165(6): 359-69.
82. Tetracycline (Code C865), National Cancer Institute, https://ncithesaurus.nci.nih.gov/ncitbrowser/ConceptReport.jsp?dictionary=NCI_Thesaurus&ns=ncit&code=C865.
83. Deeks ED. Sarecycline: First Global Approval. Drugs, 2019; 79(3): 325-9.
84. Streptomycin, Waters M, Tadi P, https://www.ncbi.nlm.nih.gov/books/NBK555886/.
85. Sarfo FS, Phillips R, Asiedu K, Ampadu E, Bobi N, Adentwe E, et al. Clinical Efficacy of Combination of Rifampin and Streptomycin for Treatment of *Mycobacterium ulcerans* disease. Antimicrob Agents Chemother, 2010; 54(9): 3678-85.
86. Keep MF, Uchino H, Elmér E. Introduction: Immunosuppressants as Neuroprotective Agents. In: Borlongan CV, Isacson O, and Sanberg PR (eds.). Immunosuppressant Analogs in Neuroprotection, New Jersey; Humana Press: 2003.
87. Paschall AV, Ozdilek A, Briner SL, Brindley MA, Avci FY. Modulation of immunosuppressant drug treatment to improve SARS-CoV-2 vaccine efficacy in mice. Vaccine, 2022; 40(6): 854-61.
88. Survase SA, Kagliwal LD, Annapure US, Singhal RS. Cyclosporin A – A review on fermentative production, downstream processing and pharmacological applications. Biotechnol Adv, 2011; 29(4): 418-35.
89. Fagiuoli S, Bruni F, Bravi M, Candusso M, Gaffuri G, Colledan M, et al. Cyclosporin in steroid-resistant autoimmune hepatitis and HCV-related liver diseases. Dig Liver Dis, 2007; 39(Suppl 3): S379-S385.
90. Li J, Kim SG, Blenis J. Rapamycin: One Drug, Many Effects. Cell Metab, 2014; 19(3): 373-9.
91. Pape E, Parent M, Pinzano A, Sapin-Minet A, Henrionnet C, Gillet P, et al. Rapamycin-loaded Poly (lactic-co-glycolic) acid nanoparticles: Preparation, characterization, and *in vitro* toxicity study for potential intra-articular injection. Int J Pharm, 2021; 609: 121198.
92. Ricci KW, Hammill AM, Mobberley-Schuman P, Nelson SC, Blatt J, Bender JLG, et al. Efficacy of systemic sirolimus in the treatment of generalized lymphatic anomaly and Gorham-Stout disease. Pediatr Blood Cancer, 2019; 66(5): e27614.
93. Moreno Altamirano L. Epidemiología y diabetes. Rev Fac Med UNAM, 2001; 44(1): 35-7.
94. Scientific Discussion, European Medicines Agency, https://www.ema.europa.eu/en/documents/scientific-discussion/humalog-epar-scientific-discussion_en.pdf.
95. Simpson D, McCormack PL, Keating GM, Lyseng-Williamson KA. Insulin Lispro: A Review of its Use in the Management of Diabetes Mellitus. Drugs, 2007; 67(3): 407-34.
96. Landgraf W, Sandow J. Recombinant Human Insulins - Clinical Efficacy and Safety in Diabetes Therapy. Eur Endocrinol, 2016; 12(1): 12-7.
97. Mbanya JC, Sandow J, Landgraf W, Owens DR. Recombinant Human Insulin in Global Diabetes Management - Focus on Clinical Efficacy. Eur Endocrinol, 2017; 13(1): 21-5.