Teachers' guide to the topic

Novel antibiotics to overcome drug resistance: A synthetic biology approach

1. Bacteria vs. viruses

Bacteria are the simplest living organisms on the earth. They are called "prokaryotes" because they are composed of a single cell without a nucleus, mitochondria, and chloroplasts. The genetic information (DNA) is arranged as a ring and located in a core-like region. Instead of mitochondria and chloroplasts, bacteria have simple protein structures, which take over the functions of energy production and photosynthesis. Bacteria multiply by binary fission. The four main types of bacteria are cocci, bacilli, spirilla and vibrios. Most bacteria are harmless to humans, but a few are responsible for such diseases as tetanus, diphtheria, tuberculosis, pneumonia, meningitis, etc.

Viruses are infectious agents consisting of only one strand of genetic material (DNA or RNA) in a protein shell. Viruses cannot multiply the way bacteria do, they need living cells as hosts instead. Viruses bring their genetic material into the cell, the cell produces new viral particles and releases them. During this process, the cell membrane is dissolved or damaged, because large parts of the membrane are taken at the exit of the virus to build up the viral shell. Viruses cause such diseases as: flu, herpes, AIDS, diphtheria, mumps, and colds.

Viruses and bacteria compared:

1. Most viruses (20-300 nm) are much smaller than bacteria (1000-5000 nanometers).
2. Viruses do not belong to living organisms, but bacteria do.
3. Viral particles have simple construction: they consist only of genetic material and a protein shell. Bacterial cell consists cytoplasm, genetic material, and cell wall.
4. Viruses need a host for reproduction; bacteria reproduce by binary fission.
5. Viruses cause a disease by destroying cells or making cells being destroyed by immune cells. Bacteria excrete toxic metabolites that cause a disease.
6. Bacterial diseases are treated with antibiotics; to fight viruses, you need antivirals.

2. Antibiotics

Antibiotics are substances that kill bacteria or inhibit their proliferation and are used to treat bacterial diseases. There are two general types of antibiotics: broadband,

which are effective against many different bacteria, and narrow-spectrum antibiotics, which can be only used against very specific pathogens.

There are several classifications of antibiotics, f.e. depending on their activity:

1. Bacteriostatic – antibiotics inhibiting the growth and reproduction of bacteria.
2. Bactericides – antibiotics killing the pathogen.

Antibiotics can be classified according to their chemical structure, a site of action, or a mechanism of action: they can inhibit the synthesis of the components of the cell wall, interfere with the bacterial DNA or RNA, and inhibit the bacterial metabolism.

Some metabolic products of bacteria, fungi or plants have antibiotic activity, but modern medicine employs only substances that are either fully synthetic, semi-synthetic or produced by means of biotechnology. Human beings and other mammals also produce antibiotics – lysozyme.

Although antibiotics are very helpful to tackle various infectious diseases, they also have side effects. A long-term antibiotic treatment or use of very strong antibiotics may cause allergic reactions, intestinal disorders (diarrhoea), or appearance of fungal infections.

Proliferation of bacteria that were not killed by antibiotics leads to the development of resistance. In such case, some or numerous antibiotics are ineffective.

3. Lantibiotics

In order to deal with the problem of antibiotic resistance, medicine needs antibiotics of last resort or entirely new antibiotics.

Lantibiotics can be a main source for the development of a new class of antibiotics, because they can inhibit bacterial biosynthesis via a novel mechanism. They can also eliminate the pathogens that are resistant to conventional antibiotics.

Lantibiotics are a group of antimicrobial peptides, synthesized by Streptococcus bacteria and containing the amino acid lanthionine. Although conventional antibiotics are synthesized in laboratory, lantibiotics can be produced by means of genetics. For each lantibiotic there is a structural gene encoding a precursor peptide. This makes it possible to modulate the anti-microbial activity of lantibiotics, or other properties in order to create specific lantibiotics that can target individual agents. Such targeted mechanism of action makes them significantly more effective than conventional antibiotics.

The mechanisms of action of lantibiotics include depolarization of the cytoplasmic membrane (type -A lantibiotics, such as nisin), which further leads to the formation of holes in the bacterial cell membrane, or inhibition of the biosynthesis of the cell membrane components (type B lantibiotics, such as cinnamycin).

4. Amino acids

Amino acids are organic acids, composed of at least two carbon atoms and containing an amino group. There are more than 400 amino acids, but only 20 of them build peptides and proteins. These proteinogenic amino acids, encoded in the genome, are: alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenilalanine, proline, serine, threonine, tryptophan, tyrosine, and valline. Some organisms have two more amino acids – selenocysteine and pirrolysine (referred to as 21^{st} and 22^{nd} proteinogenic amino acids). In order to describe amino acid, scientists use a single- and/or three-letter code: alanine – A and Ala, glutamine – Q and Gln, lysine – K and Lys.

There are eight essential (valine, tryptophan, threonine, phenylalanine, methionine, lysine, leucine, and isoleucine) and fourteen non-essential amino acids. Non-essential amino acids can be synthesized by the body itself, while the essential amino acids come from external sources with food.

The main tasks of amino acids are control of growth processes in the body, protection against diseases and toxins, and participation in the metabolism. Many amino acids act as hormones or transmitters; they participate in the biosynthesis of important molecules that ensure metabolism (coenzyme A, creatine, carnitine etc.).

In a protein molecule, the amino acids are linked by peptide bonds. This bond is formed between the carboxyl group of one amino acid and the amino group of the next one. Two amino acids form a dipeptide, three – a tripeptide, several amino acids – an oligopeptide. Amino acid chains, consisting of 10-100 amino acids, called polypeptides. Proteins usually contain more than 100 amino acids.

There are four levels of protein structure:

1. Primary structure – a simple sequence of amino acids.
2. Secondary structure – chains of amino acids, linked together by hydrogen bonds. They form α - helices or β -sheets.

3. Tertiary structure – a three-dimensional structure, formed by the folding of secondary structures. The following bonds stabilize this conformation: Van der Waals bond (between non-polar groups), hydrogen bonds (between polar groups), ionic bonds (between side chains of amino acids) and disulfide bonds (between two cysteine residues or cysteine and some other amino acids).
4. Quaternary – a supramolecular structure that contains more than one subunit of a proteine (f.e., haemoglobin, ribosomes etc.).

5. SYNPEPTIDE project

Multidrug-resistant bacteria found mainly in hospitals and nursing facilities, have developed resistance to the most antibiotics. They include methicillin-resistant Staphylococcus aureus, vancomycin-resistant enterococci, penicillin-resistant Streptococcus pneumoniae and beta-lactamase-producing bacteria (beta-lactamase is a chemical that can degrade certain types of antibiotics).

Multidrug resistance develops when antibiotics are taken longer than necessary or if they are not required. The more often antibiotics are used, the more likely it is that resistant bacteria will develop.

New types of lantibiotics could help to solve the problem of multidrug resistance. Newly synthesized peptide modules form the building blocks of lantibiotics. Scientists can combine them again to make a number of lantibiotic molecules with different properties and different types of antimicrobial activity. In addition, there are no known mechanisms that can lead to the development of resistance toward lantibiotics.

There are numerous difficulties in the production of lantibiotics. For this reason, adequate amounts of lantibiotics for clinical testing and marketing have never been produced. However, synthetic biology offers a system of organic synthesis, which can produce fully functional lantibiotic molecules in a cost-effective way.

The focus of the project SYNPEPTIDE is on the design and production of novel antibiotics based on a comprehensive synthetic biology approach. Scientists define pre-existing peptide modules from the group of lantibiotics and combine them to create new antibiotics with novel features.

All newly synthesized peptides can be modified using specific enzymes, with the nature of modifications depending on the cell type. This also applies to lantibiotic peptide modules. In order to unify these different modification pathways, the

researchers propose to use a promiscuous enzyme and organize the way of modification in a modular fashion. Thus, they will create a unique modification pathway, which could also allow fine-tuning of further modifications. This path is then installed in a bacterium (Staphylococcus carnosus) and is used to produce new lantibiotics in preparative quantities. Consistent application of the principles of modularity and context insensitivity can help create a production system of higher robustness and predictability.

This project also serves as a concrete example to explore the potential impact of synthetic biology on the safety of biotechnological processes and their ethical implications for society.