# Applications of Genetic Engineering and Biotechnology in the Pharma Industry

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## Preface

In this paper, we will discuss applications of genetic engineering in medicine, specifically pharmaceuticals.

We were very eager to expand our knowledge concerning the various applications, both useful and practical, of genetic engineering and biotechnology. Specifically speaking, we wanted to learn about the impacts of these technologies and their role in the pharma industry. Though we don't agree on all things, we both share a great interest in pharmaceuticals and their significant role in the well-being of all humans.

For over 100 years humans have been benefiting from pharmaceuticals. Since then they've undergone a vast number of changes in things like their safety and effectiveness. Not only this but also how they are manufactured and in what form they are handed over to the patients. We are especially interested in learning precisely what role biotechnology and genetic engineering play in this area. On top of this, we would like to know more about the more recent developments in this area: genetic engineering of human cells.

There was a multitude of questions that arose during our research:

- What are critical applications of biotechnology and genetic engineering for the manufacturing of pharmaceuticals?
- What are different types of applications?
- What does one specific application look like and what are it's risks and benefits?
- What benefits are there for the patients?
- What benefits are there for the pharma companies?
- What are the risks involved?
- What are future developments/What lies in the future?

## Introduction

Gene technology is a subchapter of biotechnology and deals with the isolation, characterization, and recombination of genetic materials. By targeting changes in the genetic material of living cells, new combinations of genes can be made. The (primary) result of this process is recombinant DNA. This recombinant DNA can be transferred into organisms, consequently making GMOs (genetically modified organisms) [1].

Over the past 50 years, such methods have led to some scientific breakthroughs in fields like:

- Agriculture, for instance in genetically modified crops [2].
- Pharmaceuticals [3].

As mentioned earlier, we decided to focus on the pharmaceutical aspect. Our investigations showed that there are many different applications of genetic engineering and biotechnology within the pharmaceutical industry:

1. First of all, it helped in finding **new ways of discovering drugs**: scientists are now able to discover new (potential) drugs much quicker than before with the help of, e.g. CRISPR-CAS technology [4].

2. Not only are they easier to discover, but there are now **new ways of manufacturing pharmaceuticals** by using genetically modified organisms like yeast or hamster cells[5]. This enabled the pharma industry to also produce these new drugs faster. Not only faster, but more friendly towards the environment and cheaper. This is therefore also the main focus of our term paper, see below.

3. **New types of pharmaceuticals** are being produced with the help of living cells thanks to gene engineering. These “large molecules” include new cancer medications being produced by companies like Roche and Novartis. This gives a new shimmer of hope for cancer treatment [6].

4. The last (but certainly not least) and most recent approach is the **genetic modification of living human cells in our body**. Since this is a very exciting and promising field we also wanted to learn more about this and therefore included a summary of this new technology at the end of our paper [7].

## Topic of our Project

In our project we researched and investigated the **use of genetically modified microorganisms for the production of pharmaceuticals.**

The biologically active molecules in medicines are called “Active Pharmaceutical Ingredients”, a.k.a APIs [8]. APIs can then be transformed into a number of different forms: pills, tablets, capsules, vials, ampoules etc.

There are two different kinds of APIs available on the market as of today:

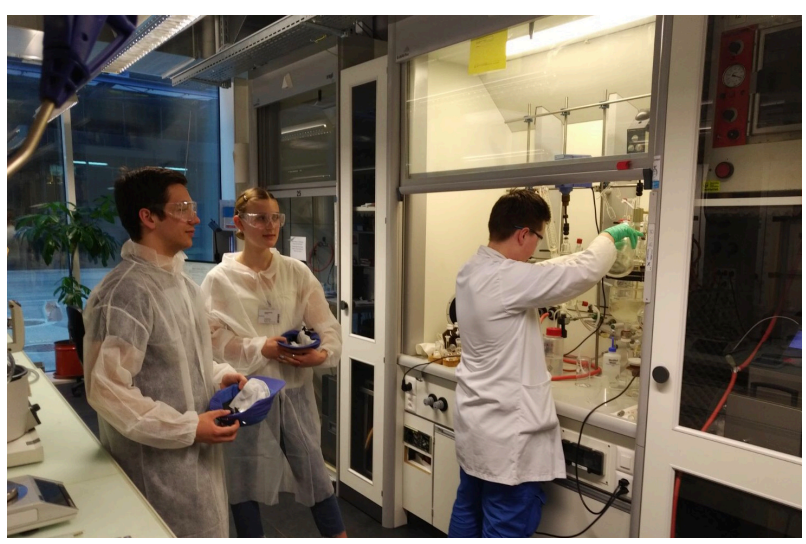
- *Small molecules*: Aspirin (pain killer), Januvia (for diabetes) etc.
  These are produced mostly by chemical reactions. They can be taken in orally and are also relatively cheap. We will be getting into this further as part of our project.
- *Large molecules*: Insulin (diabetes), Avastin (cancer) etc.
  Another name for them is *biologics*. These are mainly produced by living cells [5]. However, they do need to be applied intravenously (through a vein) and are expensive [6]. This is not part of our project and we will not be discussing these further.

As mentioned briefly before *small molecule* drugs are produced by chemical reactions. However, sometimes they are not easily produced this way. Therefore chemists and biologists developed a different way of producing them: biotechnologically.

But why are some small molecule drugs difficult to produce?
Many API’s contain so-called *chiral centers.* This means that a carbon atom has four different substituents attached to it. Molecules with these chiral centers exist as *enantiomers*.

Enantiomers are structurally like image and mirror image. This means that both (there are always two) enantiomers interact differently with our body since our body has a lot of proteins and hormones that consist of only one of the two enantiomeric forms. This is why it's important to produce APIs in only one enantiomeric form with a high purity. This is difficult and GMO's like yeast cells or E-Coli are better suited for this task than traditional chemical reagents. Nowadays there are several such GMOs and enzymes derived from it available and used for the production of pharmaceuticals [9].

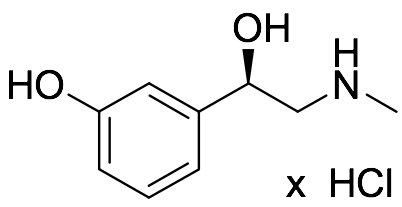
In our project we wanted to learn how companies are using genetically modified microorganisms for the production of APIs. To do this we had the wonderful opportunity to visit the company Siegfried on April 17, 2018. Siegfried is a multi-national company that produces many API's for other pharmaceutical companies around the world.



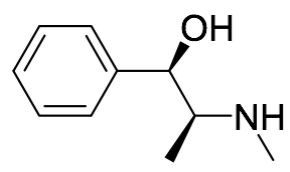
They themselves have developed various processes based on our topic for the production of pharmaceuticals. During our visit to Siegfried in Zofingen we met with their Chief Scientific Officer Dr. Wolfgang Wienand and also had a skype call with one of their researchers, Silvia Ott, at their production site Minden in Germany. We also visited their labs and a production plant. They explained two applications with us, as outlined below.

## Production of APIs with genetically modified microorganisms: Visit to the company Siegfried

Through them we were able to discuss and learn about two APIs, **L-Phenylephrine** and **L-Ephedrine**, in detail. Both of them are used as decongestants (helps you breathe easier when you have a cold):




L-Phenylephrine

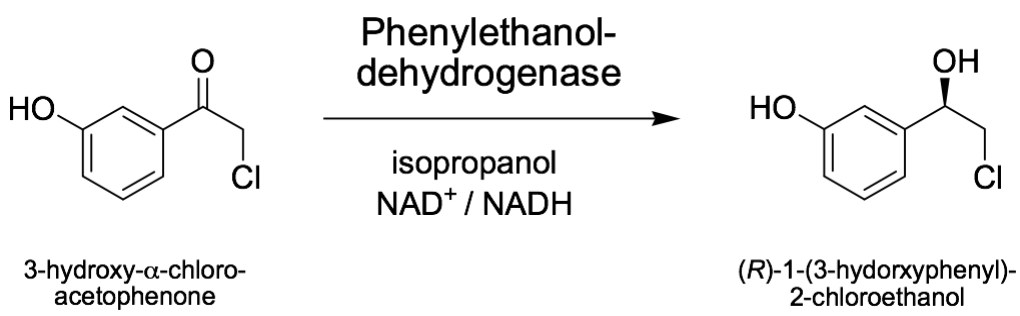



L-Ephedrine

As can be seen from their chemical formulas, both possess chiral centers and it's important to produce only one enantiomer of both. To do this in the most efficient way possible Siegfried has developed GMOs for their production.

Example 1: L-Phenylephrine

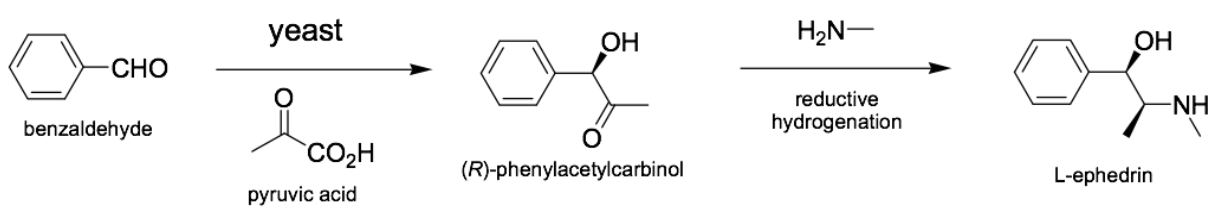
For its production an enzyme is used which reduces a keto group to an alcohol group, see reaction scheme below.




This enzyme –Phenylethanoldehydrogenase- is taken from a microorganism called E-Coli. E-Coli is a very common and abundant organism. The scientists have genetically modified this organism to get recombinant E-Coli. This genetic modification was done so that the "new" E-Coli produces much more of the desired enzyme than without any gene modification. This work was done in a special laboratory in Germany. Once the scientists had a small amount of the GMO microorganism that produced a lot of the desired enzyme, they optimized the reaction of the enzyme with the substrate (a molecule upon which an enzyme reacts) (see reaction pic).
Finally they were very successful and were able to achieve a very high selectivity. This means that there was only one enantiomer formed in the reaction. This reaction was optimized even further until it was put into practice in one of the large production plants of Siegfried in Germany. Currently they produce several tonnes per year of this medication with the help of the GMO.
We were told that this is the single best alternative to produce this medication. Of course, they filed patents for this technology in order to prevent others from using or stealing it.

Example 2: L-Ephedrine

For the production of L-Ephedrine yeast was used. However, "normal" or naturally-occurring yeast did not do a good job. It reacted very slowly and not selectively. Therefore, the scientists began modifying the yeast:  they activated mutations of the yeast's DNA by shining UV light on it. This was done many times, over and over again and the resulting yeast tested again for the reaction.
After many, many mutations they finally got a yeast strain which showed a very high and selective reactivity. Then the scientists had to do two more chemical reactions to get to the API: one with methylamine and a hydrogenation with H2, see scheme below.



Just like with the L-Phenylephrine this reaction was developed further in the laboratory and then implemented at a large production building, also in Germany. Siegfried now produces several 100 tonnes per year of L-Ephedrine.

When we first arrived we got to talk to the company's Chief Scientific and Strategy Officer Dr. Wolfgang Wienand who took us back to the basics of their biological and chemical processes. Afterwards, we were able to take a tour of the labs and factory.

Interview with **Dr. Wolfgang Wienand**, Chief Scientific Officer and Head of R&D at Siegfried:

The simple example they gave us was the addition of two hydrogen atoms to a compound called salicylic acid. This is a reaction that was extensively handled in the Schwerpunktfach chemistry module due to its importance to the synthesis of aspirin. So we asked ourselves what separated this whole industry from our lab upstairs and we received an answer consisting of two words.

Scale and catalyst.

On an industrial level, the main drive of pharmaceutical chemistry is creating the largest amount of substance at the lowest cost as efficiently as possible and there is a handful of options for their catalyst that contribute to that.

When it comes to the selection of a catalyst, pharma companies are given an array of choices which will influence the quantity and quality of their product. The chemical path would be the first option to take, one that is rarely done these days due to more effective solutions.

The next option is one that concerns us the most, and that is the use of enzymes. It is a commonly known fact that enzymes are very effective biocatalysts and much quicker than their anorganic counterparts. These are produced by genetically modifying a strain of bacteria e.g. E.Coli to produce the enzyme required, this enzyme is then extracted from the colony and produced to have the form of a usually white powder (that form speeds the reaction up the most) and they use that to catalyse the reaction. This biotechnological process is called *white biology* and the term describes the use of enzymes and organisms in syntheses of compounds. Siegfried frequently uses an enzyme from this technique called dehydrogenase.

There is yet another option at the manufacturer's disposal that also falls under the term of white biology which is the use of organisms that we touched on above. These syntheses are complex processes that rarely take less than a handful of steps, these steps are a chemists biggest fear and a biologists strongest tool. There are bacteria that can be used to create very specific drugs because they throw out the needed compounds and all it takes is for them to be fed. The strains of E.Coli and yeast and many other organisms are genetically engineered to produce exactly what is needed of them or the closest one can get to it so that only a few steps remain.

Interview with **Silvia Ott**, Researcher Siegfried Minden/Germany via video-conference:

After getting a good introduction into the world of large scale medicine manufacturing we got to interview Silvia Ott who is located in another one of their locations in Minden, Germany. She is the head of process development. At this point she dove deeply into the questions we sent her:

Question 1: **Why do you use these organisms in particular?**

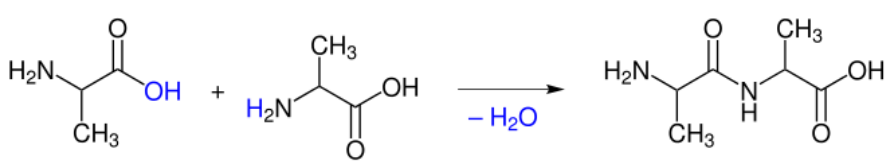
Answer 1: Ketones can effectively be synthesized into alcohols easily

Question 2: **What types of GMO do you mainly use**

Answer 2: A modified strain of the bacteria Escheria Coli

Question 3: **How do you produce these organisms? How were they genetically modified?**

Answer 3: A DNA-sequence of the Azoarcus species, which acts as a donor, is transferred to the recipient (E. Coli species). This gene produces dehydrogenase (…)




Question 4: **What security measures are in place when treating GMO's?**

Answer 4: The strain is only produced under circumstances that are never or rarely seen outside of a controlled environment. The strain does not carry any diseases that are transmittable to humans, plants, or animals. The transmission of genes with other genotypes outside of designated laboratories.

Biohazard level S1: E.Coli (…)

- All operations are conducted in accordance with the GMO Act.
- The launch and termination of all operations are notified to regional governing officials.
- Their committee for genetic engineering procedures and their doctors are informed of progress and status of all genetic engineering projects.
- All facilities are marked by biohazard level 1 markings.
- An official for biohazard safety management is in place.
- Access to facilities is only given to authorized personnel.
- A plan of action is in place, which describes safety protocol in case of a security breach, the first priority is to cut off any way for the E.Coli to enter drinking water supplies.
- A hygiene plan is in place (disinfection and skin protection)
- All GMO-containing waste is deactivated via autoclave sterilization
- GMO's are transported in safe containers
- All supplies are kept in a freezer, access is only granted to a handful of authorized personnel
- Contaminated clothes or tools are sterilized via autoclave (see graph below)
- Thermal Deactivation by Heating to 60°C

| Reaktionsdauer [h] | Lebendtiter [cfu/ mL] |
|---|---|
| 0 | >1013 |
| 0,5 | 0 |
| 1 | 0 |
| 24 | 0 |

Question 5: **Have you ever had an accident with a GMO?**

Answer 5: No.

Question 6: **How do you make sure that medication shows no traces of these GMO's?**

Answer 6: The reaction from a ketone to an alcohol is conducted in two phases (…) MTBE allows us to deactivate needless cells during the reaction. These two phases are separated post-reaction (…)

Inaktivierung durch Behandlung der GVO mit MTBE

| Reaktionsdauer [h] | Lebendtiter [cfu/ mL] |
|---|---|
| 0 | >1013 |
| 0,5 | 0 |
| 24 | 0 |

Question 7: **How much of the genetically modified substances do you use per year?**

Answer 7: ca. 800kg

Question 8: **Do these GMO's have to be produced right before use or can they store for a while?**

Answer 8: They store in Minden for about 3 months (at 4-6 degrees Celsius) after that the reaction time would be slowed down and the endeavor might not be profitable.

Question 9: **How are unused or excess GMO's exterminated?**

Answer 9: We only order as much as we need. The rest is put in containers with a 50% NaOH solution.

Inaktivierung durch Behandlung der GVO mit 1% NaOH

| Reaktionsdauer [h] | Lebendtiter [cfu/ mL] |
|---|---|
| 0 | >1010 |
| 1 | 0 |
| 2 | 0 |
| 4 | 0 |

**Conclusion of our visit**

During our visit we learned a lot about using genetically modified microorganisms and enzymes for the production of pharmaceuticals. Luckily we had the chance to talk directly to researchers. Since we did some literature studies in parallel we now know more about this important topic. We learned that genetic engineering can help to produce better medicines for people. This is good for patients who suffer from deadly diseases like cancer. It is also good for the companies and the people who work there since it increases their business. A lot of times there are no other methods available to produce such important pharmaceuticals like cancer medications.
Of course these technologies have to be applied carefully. During the interviews we learned that the researchers are very cautious when they use these technologies. They have many precautions in place when working with GMOs. They need to make sure that no such GMOs escape to the environment.

## Extra Chapter in our Project: What are the most recent applications of genetic engineering? Answer: Cell Therapy!

As mentioned in the introduction we also wanted to know what the most recent advances in the area of genetic engineering in the pharma industry are. Since we did not have an opportunity to visit a company for this we did some literature study [7] and also briefly talked with Dr. Wolfgang Wienand during our visit at Siegfried about it.

This most recent technology is called **CAR-T cell therapy**. CAR-T stands for Chimeric Antigene Receptors. With this approach patients are not treated with medications but their blood cells are genetically modified. Here, human blood cells (T cells) are taken from a patient, then genetically modified and put into the body again.
What for?
This genetic modification strengthens the white blood cells which are key players in our immune system. It uses the patient's own immune system to fight certain types of cancers. Our immune system fights imposters and harmful things like cancer cells. If we can make it stronger it can better fight harmful enemies in the body.

To summarize this technology we have attached a nice scheme we found on the homepage of the company Novartis [10]. The key steps are:

1. Extraction of T cells form a patient's blood.
2. Genetic modification of these T cells in a specialized facility. This is done by so-called viral vectors. Viral vectors play an important role in genetic engineering.
3. Grow these modified T cells, to get many more cells. We need a lot of modified cells
4. Check the quality of these modified T cells, before they are re-introduced to the patient.
5. Reduce the number of the old T cells in the blood: before the modified cells can be re-introduced into the patient, its old, un-modified T cells need to be eliminated as much as possible. This is important otherwise the new, modified cells cannot become powerful enough in the blood stream.
6. Introduce the genetically modified T cells to the patient's blood stream.
7. The modified T cell can now kill cancer cells in the body much better than the original T cells.

Being able to modify and strengthen T cells is a huge breakthrough for science. It is a new way to strengthen a patient's immune system. So far two medications have been approved for use in patients, one of them for the Swiss company Novartis. These new treatments work very well for some cancer patients. However, they are very expensive (around 500'000 USD per patient, [11]) and obviously the treatment must be closely watched since this process is very new. There might be risks we do not yet know.

We will see many new scientific discoveries in biology with many more applications in the future and hopefully one day it will be possible to successfully treat various types of cancer.

## Literature


[1] https://en.wikipedia.org/wiki/Genetic_engineering

[2] https://en.wikipedia.org/wiki/Genetically_modified_organism

[3] https://www.deutsche-apotheker-zeitung.de/daz-az/1999/daz-1-1999/uid-4444

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