MICROBIAL GENETICS

Introduction

The transfer of genetic information to the offspring during reproduction of an organism is called vertical gene transfer ("inheritance"). Eukaryotic organism, like the fungi, belonging to the microorganisms – recombine their whole genome during sexual reproduction and the individuals are characterised by their great genetic variability. Prokaryotes, which mainly reproduce by mitotic cell division (asexual reproduction), pass the unchanged genetic information on to the next generation. Additionally, to the vertical gene transfer, the genetic information can be exchanged between individuals of the same generation of a specific species or even between individuals of different species (horizontal gene transfer). With bacteria, three different transfer mechanisms can be distinguished: conjugation, transformation and transduction.

Transformation, competence

The transfer of free DNA to an acceptor cell is called transformation. If the integrated DNA provides a selection advantage, the genetic information is passed on to the next generation by means of a plasmid (after a plasmid transformation) or is implemented into the genome of the acceptor cell by recombination. The development of stable recombinants is dependent on several factors. On the one hand, the acceptor cell must have the ability for the uptake and integration of DNA (competence). On the other hand, the transforming DNA-fragments must be present as double strands, because denatured (single stranded) DNA cannot be implemented by the cells. Furthermore, the integrated DNA must be protected against restriction enzymes. These enzymes decompose "foreign", not specifically methylated and thus unprotected DNA. The DNA-methylation is different in all bacteria genera. Because of that, the transformation is possible with genetically related strains only. In the lab however, the in vitro DNA-transformation conditions are optimized in a way, in order to reach a transformation frequency as high as possible.

Restriction, recombination

If foreign DNA is intruding in a bacterial cell, two antagonistic cell systems are activated: restriction and recombination. To differentiate the own DNA from the foreign, the restriction system modifies the DNA belonging to the cell with a methylation according to a specific pattern. DNA with a different methylation pattern is recognized by the restriction apparatus as foreign and is cut by restriction enzymes. On the other hand, foreign DNA can provide a selection advantage for the cell. In this case, foreign DNA can be implemented by the recombination system into the own genome. DNA, which is as similar as possible to the own, regarding sequence and methylation pattern, is preferred.

Transduction, phages

The transfer of DNA from a donor to an acceptor cell through a bacteriophage (bacterial viruses) is called transduction. If an infected bacterium A is propagating a phage, bacterial DNA instead of phage- DNA can be packaged by chance into the protein hull of a phage particle. The infection of bacterium B however does not lead to lysis, because the necessary genetic information is not carried along by the phage. Instead, a horizontal gene transfer of DNA from bacterium A to bacterium B occurs.

Different types of transductions can be discriminated. With generalised transduction every bacterial DNA-fragment is transduced with the same (although low) frequency. In a system with specific transduction however, a phage is only transferring genes, which are immediately near the integration site of the phage-DNA in the host chromosome. If the phage-DNA is not cut correctly from the bacterial chromosome during the transition from the temperent to the virulent state, a piece of chromosomal DNA of the host bacterium gets into the protein hull of the phage particle together with the phage DNA. If more than one gene is transferred during transduction, we talk about cotransduction. The given size of the protein hull of a functional phage limits the transfer to relatively small DNA- fragments. Because of this, the frequency of co-transduction of two genes gives information about their distance on the bacterial chromosome.

IS-elements, Transposons

IS-elements (insertion sequences) are short DNA-fragments which are able to "hop around" the genetic material. They mainly consist of the genetic information, which allows these elements to cut themselves out or copy themselves and to implement on another site in the genome (or in a plasmid). Often two IS-elements form, together with an interjacent structural gene, a so called transposon. Such mobile genetic elements facilitate the gene transfer between chromosome and plasmids. If a transposon provides a useful property like a resistance to antibiotics or to heavy metals, it can propagate fast by using one of the above mentioned mechanisms.

Transformation of *Bacillus subtilis* with plasmid-DNA

Bacillus subtilis is a Gram-positive soil bacterium and has the ability to protect itself against extreme environmental conditions by the formation of an endospore. *B. subtilis* was regarded as an obligatory aerobic organism until recently. Recent investigations showed however that it can also live under anaerobic conditions.

Competency is the ability of a cell to take up DNA from the environment. There are differences between natural and induced competency. Induced competency is for example used to force *E. coli* cells to take up DNA from the environment. This is achieved by treating the cell membrane in such a way that it becomes permeable for DNA for a short period. Natural competency is observed in approx. 40 types of bacteria, which are distributed among all taxonomic groups. In most species, the natural competency is a transient condition, which can be induced by different signals, e.g. lack of nutrients or quorum sensing. Under laboratory conditions, natural competency can be induced by nutrient limitations or inhospitable growth conditions. These specific signals and the adjusting mechanisms are still unknown to a large extent. Observations, however, suggest that conditions which promote the formation of endospores, also favor competency.

<u>Aim of the experiment</u>

In this experiment, we test a *B. subtilis* strain for its natural competency using a replicative plasmid harboring a chloramphenicol resistance.

Procedure setups:

Apart from the actual transformation of the bacterium with different amounts of plasmid DNA (Setups P10, and P0) the following controls are performed:

Setup D: DNase digest

In this setup the plasmid DNA is cut into its nucleotide components by the enzyme DNase I, thereby destroying the genetic information of the plasmid

Setup K: Control of the sterility of the Plasmid DNS solution

The plasmid DNA is streaked out without bacteria. This is to check whether the DNA solution is free of chloramphenicol-resistant bacteria which could influence the results of the transformation experiment.

Material:

Per group of two students:

4 LB agar plates supplemented with 5 mg/l chloramphenicol

1 Eppendorf tube with the replicative Plasmid pHPS9

1 Eppendorf tube with sterile ddH_2O

4 Tubes

Preculture with medium I (high nitrogen) in culture tube

Tube with medium II (low nitrogen)

Per large group:

1 Eppendorf tube with DNase I solution (10 U/ml in buffer, on ice)

Drigalsky spatulas

Procedure:

The experiment is performed in groups of 2 students.

A pre-culture in medium I has been prepared to you by an assistant.

1. Prepare the setups according to the following pipetting scheme.
    a. Pipette the different setups in tubes
    b. Use a new sterile pipette tip for each individual pipetting step.

	P 10	**P 0**	**D**	**K**
DNA (plasmid pHPS9)	10 µl	0 µl	10 µl	10 µl
H2O	0 µl	10 µl	0 µl	500 µl
DNase	0 µl	0 µl	10 µl	0 µl

2. Let tube with **setup D** incubate at 37 degrees for 5 min.
3. Mix 1 ml of pre-culture with medium I to 4 ml media II
4. For all cultures above except setup K add 500 µl of the diluted culture.
5. Incubate the cells in 37°C and 100 rpm for 90 min
6. Centrifuge cells (max. rpm for 2 min) and take off 450 ul of the supernatant.
7. Resuspend re-suspend the 5 pellets in the remaining 50 ul
8. Plate the cells on provided chloramphenicol supplemented LB plates using a Drigalsky spatula in the laminarhood
    a. Remember to label your plates

9. Incubate the plates for at least 2 days in an incubator at 37°C.

Results:

	P 10	P 0	D	K
Number of colonies				

The outcome of the experiment will be analyzed and discussed in the lecture on October 24th