Student Protocol
Escherichia coli contra *Bacillus subtilis*

By DNA-sequencing it`s easy to figure out the species of bacteria you are working with. By conducting microbiology experiments it`s also possible to figure out if you are working with *Bacillus subtilis* or *Escherichia coli*. Both bacteria could be detected by their different abilities and metabolism activities.

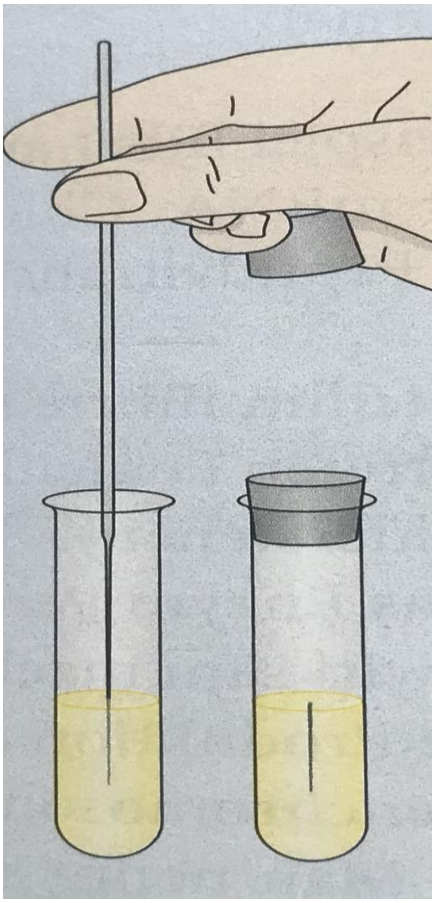
For background information *E. coli* lives in symbiosis with humans. 1 kg of *E. coli* round about 100 billion cells (10^{14}) are living in the colon of a human, best supported by food, water and optimal temperature at 37 °C. *Bacillus subtilis* lives in nature on the surface of hay and so it`s also called the hay bacteria. Both bacteria are looking quite similar under the microscope but are different in their oxygen need, cell wall structure and in their starch metabolism.

1. Oxygen Need

Materials for each work group:
Overnight cultures of *Bacillus subtilis* and *E. coli*, 2 LB-Agar (solid nutrient) in glass test tubes stoppered with cotton, 2 sterile plastic inoculation loops, gas burner;
For all groups: incubator 37 °C, 2 weighs, 2 lab spatula, H_2O_{dist}. (10 L), 8 x 200 mL Schott flasks, microwave.

Protocol:
Experiment Step 1: Preparing two petri dishes containing sterile nutrient LB-Agar
Perfect concentration of LB-Agar nutrient is 40 g of LB-Agar powder in 1 L H_2O_{dist}.
Each of your two glass test tubes should contain 15 mL of LB-Agar solution.
Because of loosing water during sterilization by evaporation calculate a total volume of LB-Agar for your tubes of 40 mL.
Weigh out the total mass of calculated LB-Agar powder and dissolve it in 40 mL of H_2O_{dist}.
Both tubes containing the nutrient are sealed with a cotton stopper and sterilized in the heat steam cooker.

Step 2: Conducting the experiment
With a sterile inoculation loop a bacteria colony is picked from the overnight culture of *E. coli*.
The inoculation loop with *E. coli* attached on it is pierced 6 cm deep into the solid LB-Agar of one glass test tube.
With a second sterile inoculation loop *Bacillus subtilis* is picked from the overnight culture plate.
The inoculation loop with *Bacillus subtilis* on it is also pierced 6 cm deep into the solid LB-Agar of the second glass test tube.



Both glass test tubes are incubated at 37 °C for 48 hours.

Tasks:
1. After incubation both glass test tubes are investigated to figure out where the growing bacteria are visible.
2. Explain the growing behavior of both bacteria regarding to their native places of living.
3. Figure out advantages for humans to live in symbiosis together with *E. coli*.

Student Protocol
Escherichia coli contra *Bacillus subtilis*

2. Metabolism of Starch

Not each bacteria species is able to metabolize starch depending on the native life location of the bacteria. To detect starch is possible with Lugol solution (Iod/Potassiumiodide-solution) which stains starch blue or black if high concentration of starch is available in the test.

Materials for each work group:

Over night cultures of *Bacillus subtilis* and *E. coli*, 2 plastic petri dishes containing LB-Agar (solid nutrient) with 2 % starch added, 2 sterile inoculation loops, gas burner, 10 mL Lugol-solution (Iod/potassiumiodide), 2 stripes of parafilm, water proofed pen.

For all groups: Incubator 37 °C, 2 weighs, 2 lab spatula, $H_2O_{dist.}$ (10 L), 8 x 200 mL Schott flasks, microwave.

Protocol:

Step 1: Preparing 2 petri dishes containing sterile LB-Agar nutrient and 2 % of starch.

To prepare a petri dish (5 cm in diameter) pour 15 mL of sterilized nutrient in it. Because of loosing water during sterilization by evaporation calculate a total amount of LB-Agar for your petri dishes of 40 mL.

Concentration of LB-Agar nutrient is 40 g of LB-Agar in 1 L H_2O_{dist}.

Weigh up the total mass of calculated LB-Agar powder and dissolve it in 40 mL of H_2O_{dist}.

Weigh up the mass of 2 percent starch out of 40 mL total mass. Add starch to your 40 mL of LB-Agar suspension and sterilize it in the heat steam cooker. Pour the liquid nutrient into your 2 plastic petri dishes. Open petri dishes only partly to avoid contamination. Let the nutrient cool down to room temperature and it will get solid and ready to use.

Step 2: Paint a smiley of bacteria on sterile LB-Agar medium containing 2 % of starch.

With a sterile inoculation loop a bacteria colony of *E. coli* is picked from the overnight culture.

Paint with the inoculation loop *E. coli* attached a smiley on the surface of the starch containing LB-Agar nutrient plate.

Pick a *Bacillus subtilis* colony with an inoculation loop from the overnight culture petri dish. Paint another smiley on the second prepared petri dish containing a 2 % starch LB-Agar nutrient.

Both petri dishes are incubated at 37 °C for 48 hours.

Evaluation of the experiment:

After 2 days incubation add 5 mL of Lugol-solution in each petri dish with the smileys.

Tasks:

1. After adding 5 mL of Lugol-solution to both petri dishes figure out where the bacteria are growing and where they could metabolize starch in the nutrient.
2. Reflect your watched results about starch metabolism regarding to the native living location of both bacteria and the existence of starch at their living location in nature.

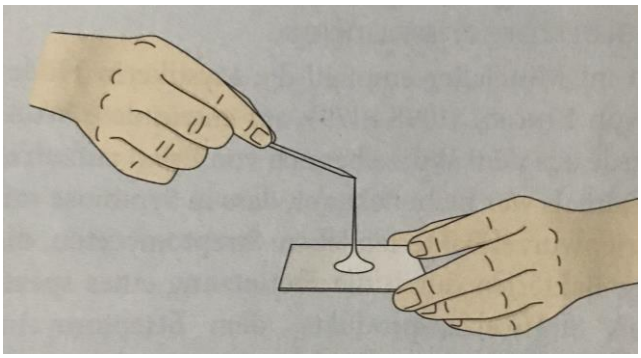
Student Protocol
Escherichia coli contra *Bacillus subtilis*

3. Gram Quick Test

By Gram staining test the thickness of the cell wall classifies bacteria in two groups: Gram positive and Gram negative species. Gram positive bacteria have a thick cell wall which could uptake the Gram stain. Gram negative bacteria have a thin cell wall which couldn`t be stained by Gram.

Materials for each work group:
Over night cultures of *Bacillus subtilis* and *E. coli* on LB-Agar petri dishes, 2 inoculation loops, 1.5 mL KOH (2 %) in a 2 mL Eppi, 2 wooden toothpicks, 2 glass object plates, single use pipet

Protocol:
With a single use pipet one drop of KOH (2 %) is made on an object plate.



With a toothpick some *E. coli* colonies are picked and transformed into the KOH drop on the object plate and stirred inside the drop. Try to take stitches out of the KOH-*E.-coli*-suspension with the toothpick.
Repeat the procedure with colonies of *Bacillus subtilis* and a drop of KOH on your second object plate and try to take stitches again with the second toothpick out of your KOH-*Bacillus-subtilis*-suspension.

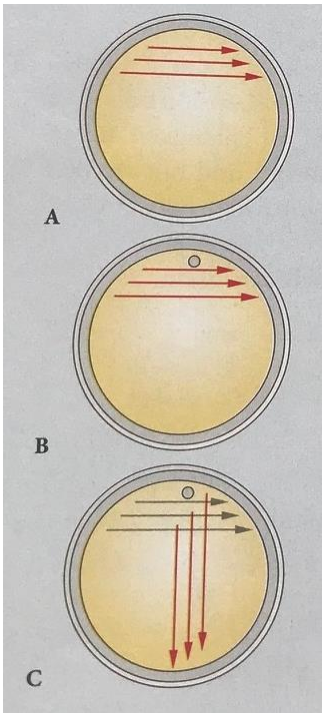
Tasks:
1. Investigate the two bacteria suspensions if it is possible to take stitches with the toothpick.
2. Explain the meaning of the KOH solution in the experiment.
3. Build a hypothesis what kind of chemicals the stitches are made out of.
3. Build a hypothesis why the Gram quick test could substitute the time consuming Gram test.

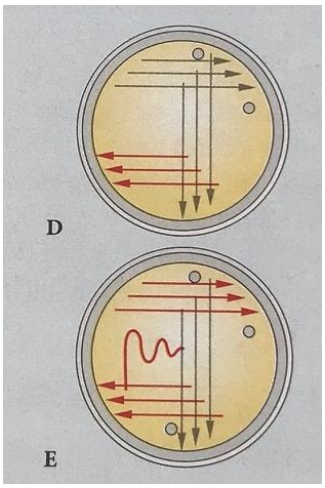
4. Production of a pure culture

To produce a pure culture consisting out of millions of bacteria but only of one specie the following procedure could be done.

Materials for each work group:
Overnight cultures of *Bacillus subtilis* or *E. coli*, 2 plastic petri dishes containing LB-Agar (solid nutrient), gas burner, 2 stripes of parafilm
For all groups: Incubator 37 °C, 2 sterile metal inoculation loops

Protocol:





Follow the steps A to E with your inoculation loop.

Only at the beginning of step A bacteria are brought in the petri dish.

Before each step B to E the inoculation loop is heated to kill all bacteria attached on it.

Tasks

1. Conduct steps A to E and incubate your petri dish at 37 °C for 48 hours.
2. Figure out where the pure cultures are located after incubation.