E. 8 Rumen Fluid Experiments

10.07.2014

Oli

In order to check whether *E. coli* and *M. capsulatus* are able to simply grow on the nutrient which are available in the rumen of cows, agar plates based on rumen fluid are prepared.

50 mL rumen fluid is centrifuged at 4000 rpm for 30 min. The supernatant is again centrifuged at 17000 rpm (34000g) for 90 min. The supernatant is firstly sterile filtrated with a 450 nm filter and afterwards with a 200 nm filter.
Additionally an agar stock solution is prepared: 50 mL water + 4 g of agar are autoclaved.

15.07.2014

Oli

Preparation of rumen fluid plates:

> 25 mL of sterile filtrated rumen fluid (see 10.07.2014) is added 10 mL of the agar stock solution = 6x small rumen fluid based agar plates.
>
> ➔ Pre-warm the rumen fluid, otherwise clots will form when added to hot agar!

One plate is incubated at 37°C without any inoculation to check weather the plates are truly sterile.

16.07.2014

Oli

After one day of incubation, the plate shows no colonies .

17.07.2014

Oli

Still no growth of colonies on the plate.

21.07.2014

Oli

Still no growth on the plate. But the plate dehydrated and is now discarded.

> ➔ The prepared sterile rumen fluid is indeed sterile. It can be used for further experiments.

25.07.2014

Oli

20 µl of competent cells are plated on a new rumen fluid agar plate, in order to check weather E. coli is able to grow on a plate based on rumen fluid. The plate is incubated at 37°C for a couple of days.

28.07.2014

Oli

No growth of cultures is observed… **Why is *E. coli* not growing on the plates, even though it is observed in the cows rumen?**

01.08.2014

Niels, Oli

The reason why *E. coli* is not growing on the prepared rumen fluid plates might be the lack of a C-source. Therefore glucose is added to one of the prepared plates: 1mL 2YT medium + 50 µL glucose stock -> added on the plate and soaked up by the plate.
Inoculation of this **rumen fluid + glucose plate** with *E. coli* cells.

04.08.2014

Oli, Rüdiger

The rumen fluid + glucose plate does still not show growth of bacterial colonies. The missing C-source was therefore not the reason for the lack of growth of *E. coli.* There must be some other inhibiting substance in the rumen fluid or maybe the pH value is responsible. For that reason different dilutions of the rumen fluid will be prepared and the pH value will be determined.

Preparation of new sterile rumen fluid for further experiments. Prepared on the same way as on 10.07.2014.

14.08.2014

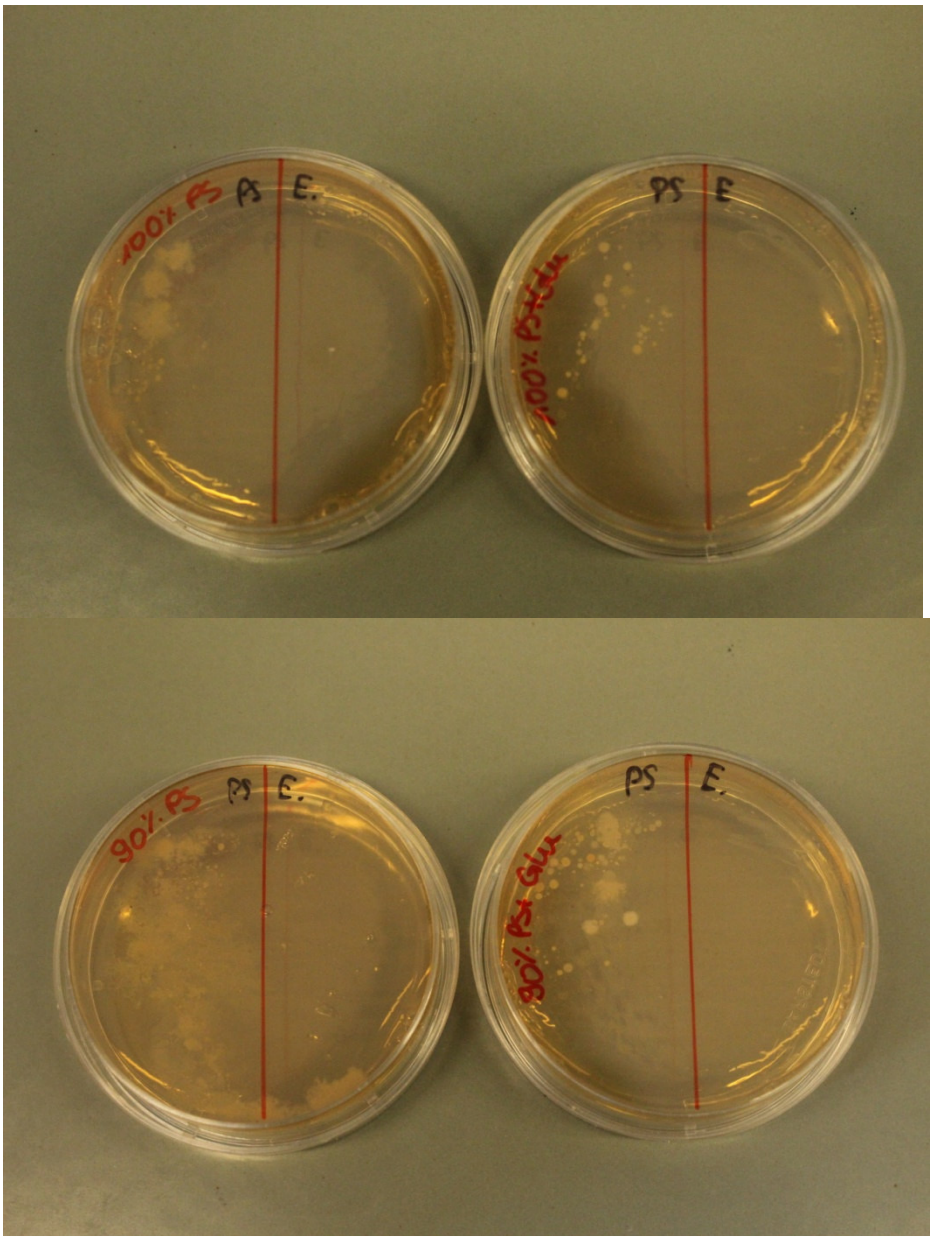
Oli, Anna, Rüdiger

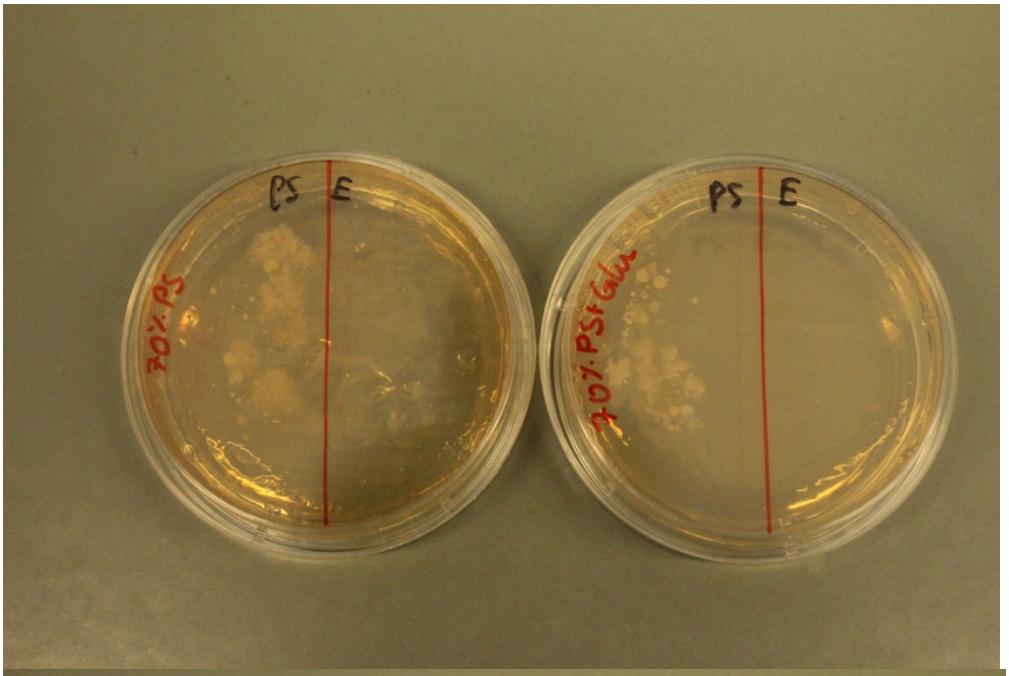
Preparation of diluted rumen fluid agar plates: 100%, 90%, 70% 50%, 30%, 10%, 0% with and without glucose (100mM) without any antibiotics. Dilution with sterile filtrated tap water. Tap water was chosen because it still contains ions which might be necessary for bacteria growth.

Preparation: 5 mL diluted rumen fluid solution + 2 mL agar stock (+350 µL glucose)

pH was determined to 7 in undiluted rumen fluid and in all dilutions using pH-paper with a precision of 1.

All plates are inoculated with E. coli (containing an empty pSB1C3 vector) and rumen fluid (unsterile).

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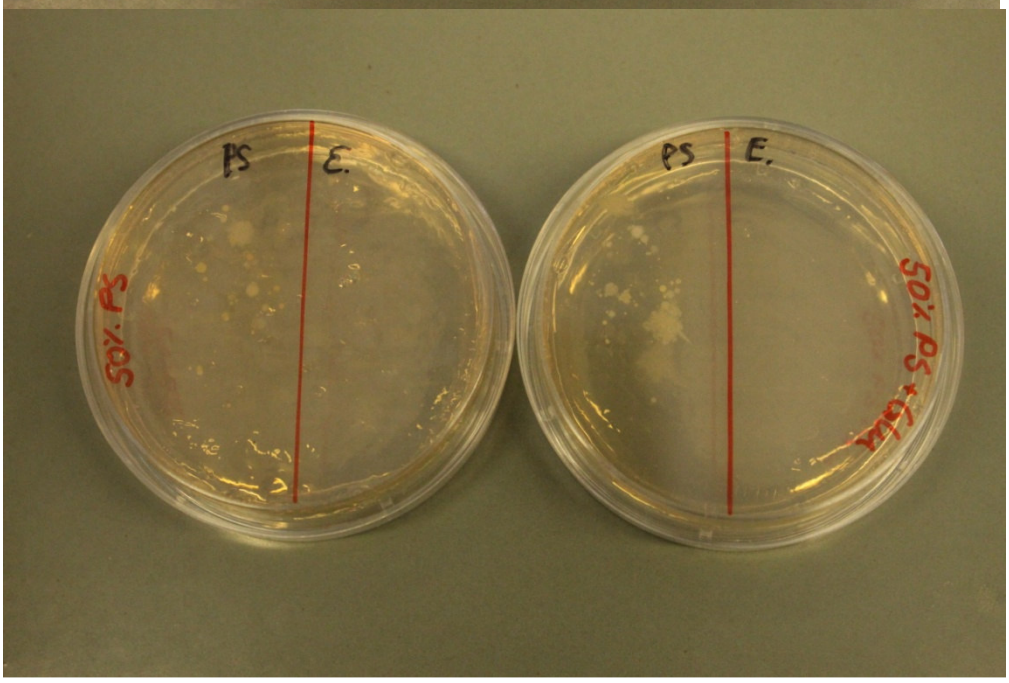
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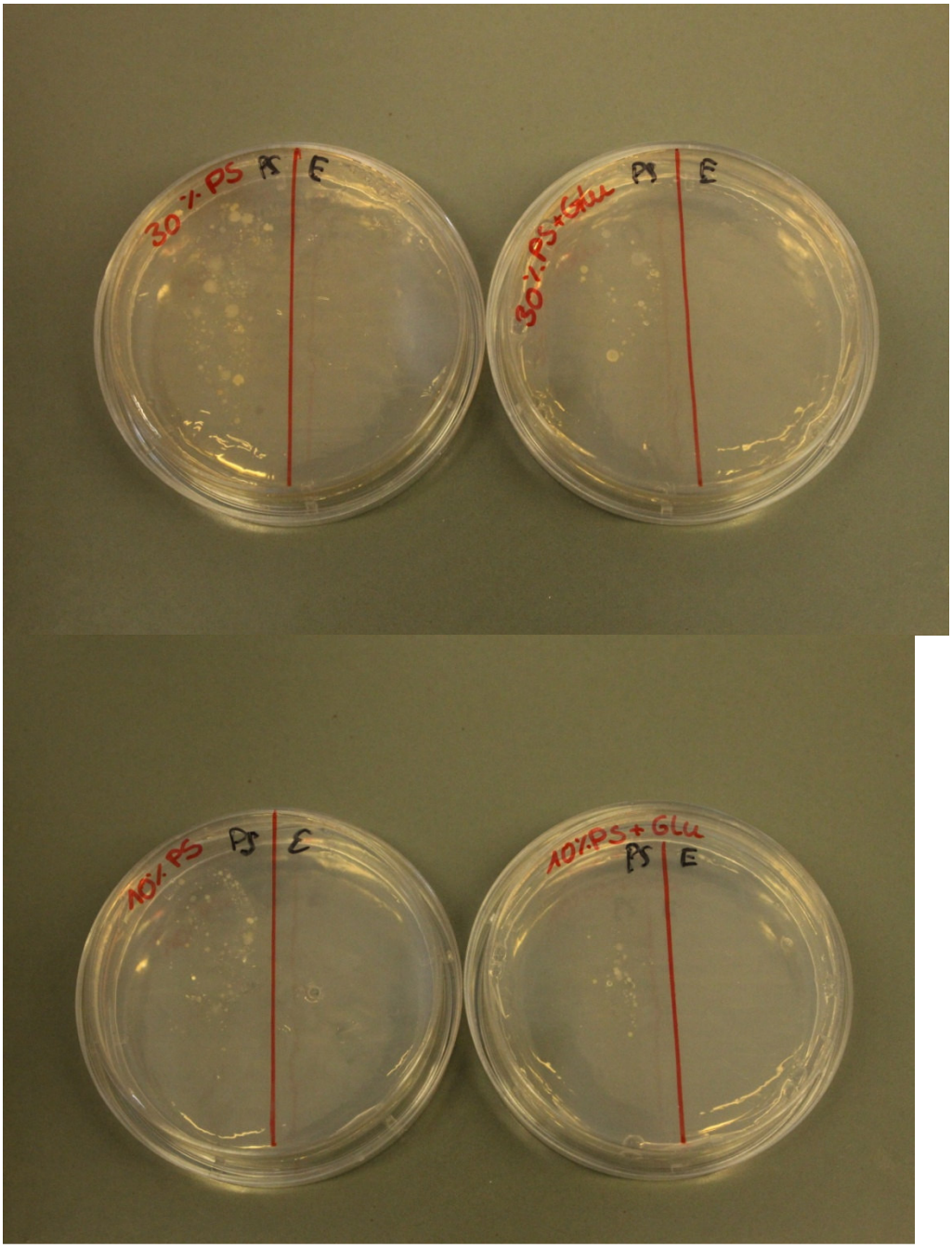
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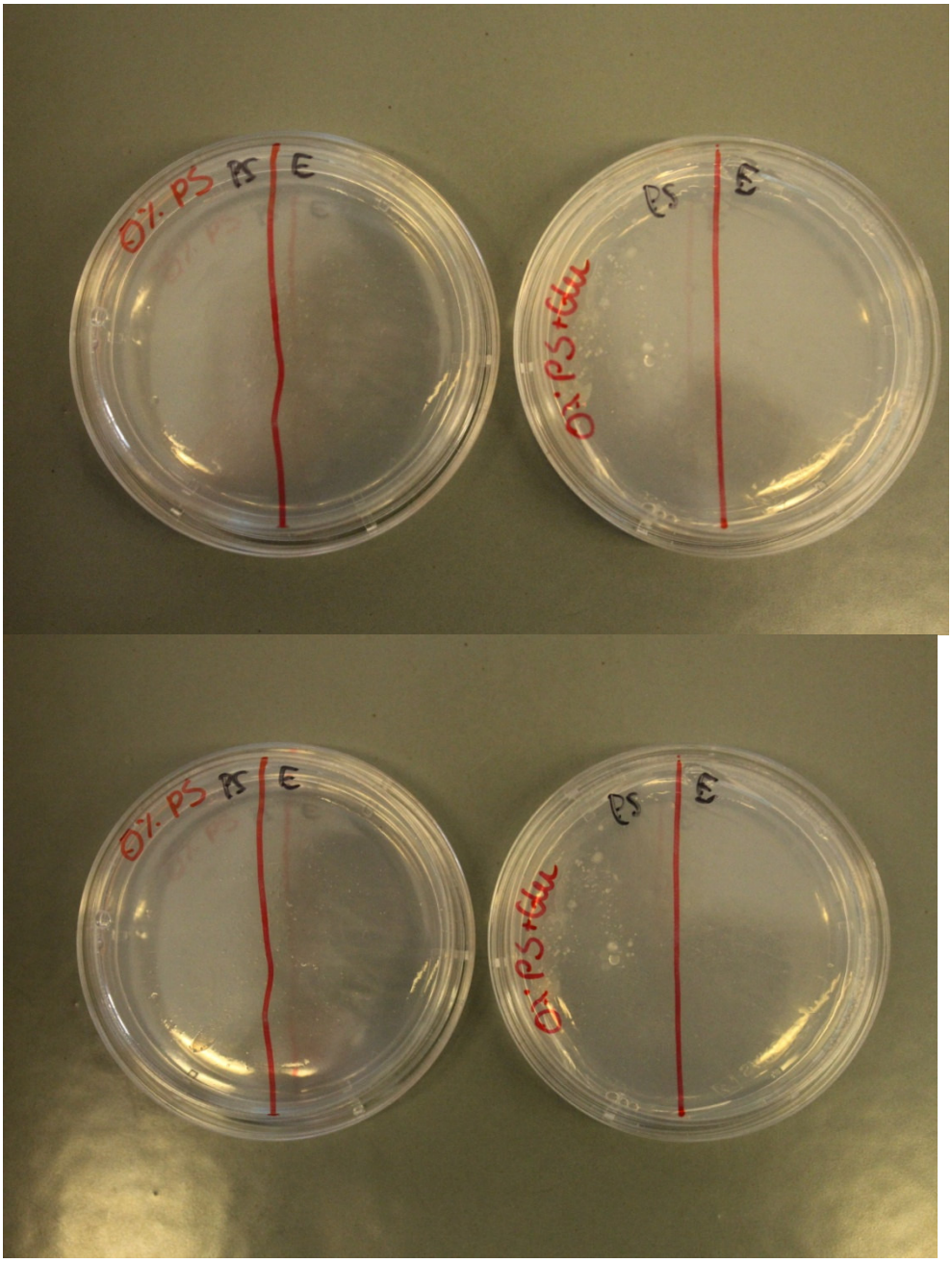

→ The experiment shows that microorganisms that normally live in the rumen can still grow on rumen fluid after it has been sterile filtrated, but *E. coli* somehow cannot utilize the nutrients provided in the rumen fluid or it is inhibited by other substances in the rumen. We will therefore provide some environment by which *E. coli* is protected from the impeding influences in the cow's rumen.