Healthy gut ecosystems

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Organisation

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SCOPE OF THE METHOD

The Method relates to	Animal health, Human health
The Method is situated in	Basic Research, Translational - Applied Research
Type of method	In vitro - Ex vivo

DESCRIPTION

Method keywords

gut

bioreactor

ex vivo

gut microbiota

cecum

Scientific area keywords

microbiology

antimicrobial resistance

probiotics

prebiotics

digestion

Method description

With a one vessel fermentor (BioFlo 110, New Brunswick), an *in vitro* simulation of a certain part of the gastrointestinal tract (e.g. cecum, colon) is performed. In this fermentation system, physiological gut parameters (pH, temperature) are kept constant, while chemico-physical characteristics of the gut chime are simulated by adding pork mucin and fibre to the fermentation medium. For a first screening, the fermentation system can be performed in short time (24-48 h) batch mode. The system is inoculated with cecal or fecal material from animals or humans, and can be inoculated with a pathogen like Salmonella. Batch fermentations are monitored by periodic sampling on a quantitative level by dilution plating on selective media for certain bacterial groups (e.g. lactic acid bacteria, bifidobacteria, Enterobacteriaceae, clostridia) as well as for Salmonella, and on a qualitative level for the total microbiota by cultivation independent 16S rDNA based metagenomics; production of short chain fatty acids is measured by GC. From these batch fermentations, for more detailed investigation long-term (up to 2 weeks) a continuous fermentation system is used which simulates even better the gastrointestinal tract of monogastrics like the pig because it takes into account the retention time (or conversely flow of intestinal content) in a certain part of the gut. A continuous fermentation is started in batch mode as described above after which fresh medium is added with a peristaltic pump to the fermentor vessel at a fixed dilution speed and fermented medium is wasted with the same speed to maintain the medium volume in the vessel. After a few days, a steady state is reached and bacteriological and fatty acid parameters can be monitored over several days (or weeks) as described above. All fermentations (batch and continuous) are performed at least twice and for every condition or component, a blank fermentation is included.

Lab equipment

1 liter bioreactors with peristaltic pumps for in and outflow of medium ;

L2 lab ;

Incubators ;
-80°C freezer ;
UPLC-MS/MS.

Method status

History of use
Internally validated
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

No animal experiments are needed to investigate the gut microbiota ;
Ease of sampling and reproducible conditions can be executed ;
Two modes of operation are possible depending on the time and resources available :
1/ batch fermentation for screening is possible in a few days ;
2/ continuous fermentation is possible for more in depth studies, taking a few weeks.

Challenges

The *ex vivo* system only simulates the gut luminal microbial ecosystem without an animal or human host component (except for addition of porcine mucin to the medium) like gut epithelial cells and host immune system.

Modifications

Addition of mucin beads is possible for simulation of attached bacteria to the mucin layer.

Future & Other applications

Applications in biofilm research are possible, both in the gut ecosystem as on inert surfaces.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

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Associated documents

Campy-allicine.pdf

Campy-Enterococcus screening.pdf

ESBL conjugatie.PDF

Salmonella-in vitro.pdf

Selection and transfer of an IncI1-tet(A) plasmid.pdf

cross contamination doxycycline ex vivo model.pdf

Links

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