In vitro digestion, absorption and colonic batch fermentation model

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SCOPE OF THE METHOD

The Method relates to	Animal health, Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	Pigs, humans
Type of cells/tissues/organs	Fecal samples

DESCRIPTION

Method keywords

intestinal microbiota

short-chain fatty acids

gas production

batch fermentation

fermentation supernatants

enzymatic hydrolysis

dialysis

Scientific area keywords

intestinal absorption

colonic fermentation

batch model

three-step in vitro pig gastro-intestinal tract model

digestion

intestine

Method description

The feed undergoes an *in vitro* enzymatic hydrolysis simulating the digestion in the upper digestive tract followed by a dialysis mimicking the intestinal absorption. Finally, an *in vitro* batch fermentation is performed and simulates the fermentation occurring in the hindgut. The ingredients, ground to a 1 mm-mesh screen, are first hydrolysed with porcine pepsin in a phosphate buffer supplemented with chloramphenicol (pH 2, 39°C, 2 hours) under gentle agitation in a water bath and then with pancreatin (pH 6.8, 39°C, 4 hours) in the same environment. Then, the hydrolysed samples are dialysed in standard regenerated cellulose membranes (6-8 kD) during 24 hours under agitation and water renewal to optimise the dialysis and are thereafter lyophilized. A faecal inoculum is prepared with a buffer solution (pH 6.8) composed of salts and minerals and frozen pig faeces (2.5%). An alternative to frozen faeces is to collect the faeces directly in syringes, to remove all the air and to place the syringes in a water bath at 39°C for transportation to the laboratory. The faeces and buffer solution

need to be subjected to mechanical pummelling using a stomacher and then filtered through a 250 µm-mesh screen. The fermentation starts by adding 15 ml of faecal inoculum to 0.1 g of dried hydrolysed sample in 60-ml glass bottles filled with 3 mucin carriers making up approximately 1 g of mucus-agar and closed with a rubber stopper and crimp-caps to ensure steady and continuous anaerobic conditions. These steps are performed in an anaerobic chamber. The sealed vials are then placed in a water bath at 39°C. The released gas volumes are repeatedly recorded over 72 hours of incubation with a manometer measuring bottles' inner pressure or with pressure sensors and connected to a computer. Gas production recordings are fitted to a mathematical monophasic model. The fermentation broth is collected and stored at -20°C for metabolomics analyses and at -80°C for microbial genomic DNA analysis. The concentrations of short-chain fatty acids, branched chain fatty acids and lactic acid can be determined by HPLC or the whole metabolomics profile can be obtained by GC-MS. Bacterial communities can be determined by qPCR or 16S sequencing.

Lab equipment

Laboratory glassware ;

Glass vials (fermenters) ;

pH meter;

Stomacher ;

Liquid nitrogen ;

Gas bottles (nitrogen and special mix for the anaerobic chamber) ;

Plastic microcosms (for the preparation of the mucin carriers) ;

Freeze-drier ;

Cellulose diffusion membrane ;

Anaerobic chamber ;

Water bath ;

Manometer ;

HPLC or GC (for the determination of short chain fatty acids and other metabolites) ;

qPCR or 16S sequencing (for the determination of the bacterial communities).

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

1) Fast, easy to set up, reproducible, high-throughput and automatable ;

2) Limited ethical and economic constraints ;

3) Valuable tool for the initial investigation of the microbial modulation and metabolic activities of a wide range of feed substrates.

Challenges

The main drawback of the method is the rapid substrate depletion, the accumulation of metabolites and the pH reduction which, in turn, can impede microbial activity.

Modifications

The method could be further optimised by implementing the selective absorption of nutrients after the hydrolysis step. Indeed, some free mono- and disaccharides and short-chain polymers are lost during the dialysis step due to the pore size of the cellulose membrane (8000 Da). Therefore, the application of the hydrolysate residues onto intestinal epithelial cell culture transwells could allow the active and passive absorption of nutrients (via their transporters) and to obtain a digesta in the basal compartment which could, more closely, simulate intestinal conditions.

Future & Other applications

A common limitation of the fermentation model is the lack of host response. This could be overcome by combining the *in vitro* fermentation system with intestinal cell models which would allow the assessment of the immunomodulatory and barrier-enhancing properties of substrates.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Uerlings, J., Schroyen, M., Bautil, A., Courtin, C., Richel, A., Sureda, E. A., … Everaert, N. (2019). *In vitro* prebiotic potential of agricultural by-products on intestinal fermentation, gut barrier and inflammatory status of piglets. British Journal of Nutrition, 1–37. https://doi.org/DOI: 10.1017/S0007114519002873.

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