Yakult

Science for Health

1495 UNIVERSITY OF ABERDEEN

The Rowett Institute

MODEL SYSTEMS

SERIES TWO: FUNDAMENTALS OF RESEARCH

Created in partnership with
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Investigating the role of any ingested product (food or drug) on human health mediated through action of the gut microbiome requires considerable research using a variety of different model systems of different complexity.

This resource:

- **Explores the advantages and disadvantages of each model**
- **Explores the role of *in silico* computer modelling**
- **Outlines the various sample collections necessary for probiotic research and how to assess the quality of study designs when reviewing the literature**

MODEL SYSTEMS

There is a natural flow through the different model systems as shown on the following page, but not all steps are essential, and information gained in subsequent steps can be used to refine and repeat an earlier model.

GENERAL FLOW THROUGH MODEL SYSTEMS

	TECHNIQUE / MODEL	WHAT TO EXPECT
IN SILICO MODELLING		
BACTERIAL CULTURE	PURE BACTERIAL CULTURE	• Culture of single bacterial strain • Used to assess bacterial growth on specific substrates and formation of metabolic products • Provides detailed information on specific growth abilities and products of individual strains • Used to identify bacterial species and/or typing individual strains • Used to test resistance to acidic conditions, bile salts, digestive enzymes, antibiotics, etc.
BACTERIAL CULTURE	DEFINED MIXED CULTURE	• Suitable only for a mixture composed of a limited number of strains • Assess bacterial interactions during growth on specific substrates • Assesses incidence of and products released during bacterial cross-feeding • Determines whether one bacterium produces compounds that inhibit the growth of another
IN VITRO MODEL	MIXED ECOSYSTEM	• Use single faecal inoculum containing entire microbial community to assess effects of specific added compounds in competitive ecosystem • Use defined mixed culture community comprising a selected mixture of pure strains • Use batch culture or continuous flow fermentation system • Determines bacterial competition for specific growth substrates and/or ratios of end-products • Allows comparison of multiple additives on human microbiota, in a controlled way
IN VITRO MODEL	CELL LINE MODEL	• Measure ability of specific bacteria/nutrients to interact with human cells and release e.g. pro/anti-inflammatory molecules • Test ability to protect or fortify intestinal barrier, adhesion potential or hydrophobicity of potential probiotic strains • Allows testing mechanisms of action e.g. using knock-out cell lines
EX VIVO MODEL	ORGANOID CULTURE	• Measure ability of specific bacteria/nutrients to interact with human cells • Can measure changes at both apical and basolateral surface • Can use biopsy tissue or tissue from biorepositories • Allows for realistic 3D studies
IN VIVO PRECLINICAL	ANIMAL MODEL	• Interaction of specific bacteria/nutrients *in vivo* • Can investigate systemic effects • Allows study of mechanisms using knock-out animals • Allows study of multiple readouts (immunological, biochemical, histological, etc.)
IN VIVO CLINICAL	HUMAN STUDY	• Intervention study in target study population • Incorporate multiple possible endpoints • Allows exploration of responder/non-responder hypotheses • Enables placebo-controlled studies

Underlined text: Refer to glossary (page 5) for definitions.

GENERAL FLOW THROUGH MODEL SYSTEMS

	ADVANTAGES	DISADVANTAGES
PURE BACTERIAL CULTURE	• Enables simultaneous testing of multiple bacteria-substrate combinations (100s) • Provides characterisation of specific growth abilities and products of individual bacterial strains	• No interactions with other bacteria or host cells • Can't apply results directly from pure to mixed culture systems nor to an *in vivo* situation
DEFINED MIXED CULTURE	• Enables comparison of many bacteria-substrate combinations • Tests bacterial interactions (cross-feeding, competition, inhibition)	• No interactions with host cells • Number of bacteria to be included depends on the growth medium/conditions • Information not immediately applicable to *in vivo* situation
MIXED ECOSYSTEM	• Tests bacterial interactions • Measure changes in microbiota composition and products • Whole ecosystem present • Can include mucin layer • Variable advantages of Batch Culture and Fermentor Systems*	• Limited number of substrates • Low throughput • No absorption, no immune or endocrine parameters • Batch: substrate limited, toxic products accumulate • Lower reproducibility
CELL LINE MODEL	• Measures interactions with host cells • Detect expression e.g. pro/anti-inflammatory molecules	• Limited number of bacteria/substrate combinations • Low throughput, time consuming • No absorption • Laboratory-adapted cell lines
ORGANOID CULTURE	• Measures interactions with host cells • Detect expression of molecules of interest • Simulates absorption • Can use biopsy tissue from healthy/diseased sites	• Limited number of bacteria/substrate combinations • Low throughput, time consuming • No absorption
ANIMAL MODEL	• Used to confirm previous data/test hypotheses on mechanism of action (MOA) • Ensures absorption and circulation of products around body • Usually use rodent model • Legally necessary for safety tests of new products	• Limited number of tests possible • Not always necessary or appropriate (ethical considerations) • Animal models not 'same' as humans (different physiology and microbiota) • Can't directly translate findings to target host
HUMAN STUDY	• Used to confirm findings in target human host • Can investigate effects on additional parameters • Allows long-term testing in healthy (food) or short-term testing in diseased populations (drugs)	• Interindividual variation may mask effect • May require a large number of participants to show real effects • Problems of responders and non-responders

It is crucial to remember that a model remains a model – and results can never simply be extrapolated to the human system without confirmatory studies!

*BATCH CULTURE SYSTEM

- Closed system
- Higher throughput (10s of combinations)
- Short timescale (24/48 hr)
- Limited by metabolite build up (potentially toxic and acidic pH) and substrate depletion

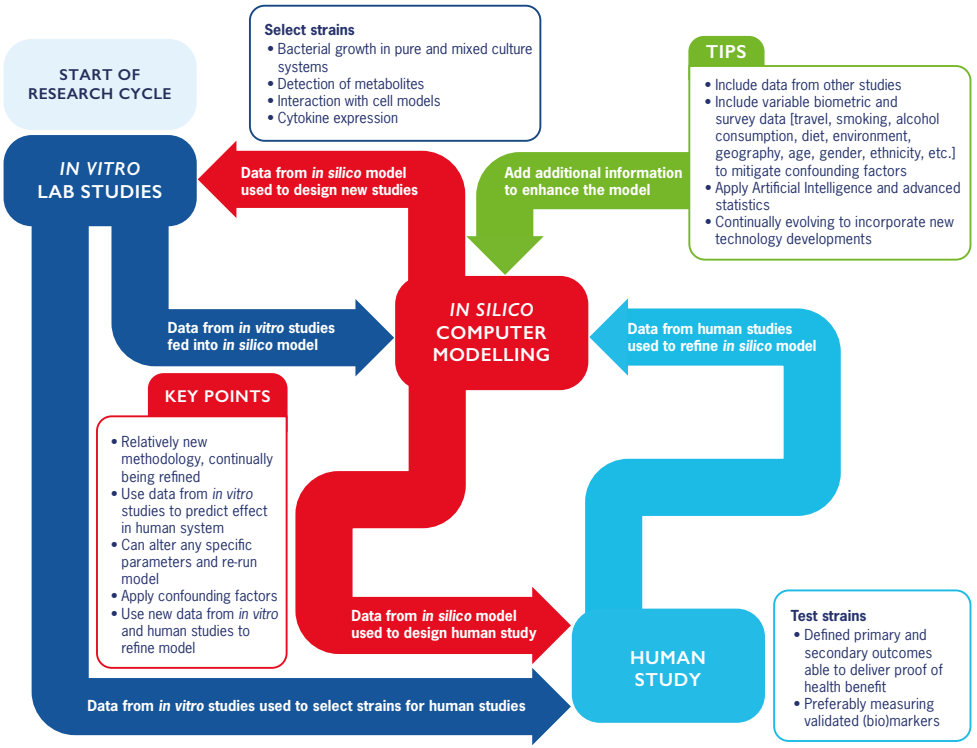
*CONTINUOUS FLOW FERMENTOR SYSTEM

- Flow through system
- Smaller reproducibility (e.g. 4 donors, 6 compounds)
- Longer time scale (up to 2 weeks or more)
- Continual input of substrate and removal of metabolite products

Underlined text: Refer to glossary (page 5) for definitions.

USING MODEL SYSTEMS IN PRACTICE

Remember using model systems is not a linear process. This flow diagram shows how information gained in one model can be used to refine and repeat an earlier study or inform a subsequent study which uses a different model.



*For more information on **Human Studies**, please see resource **Skills Series 2: Fundamentals Of Research - Human Studies.**

GLOSSARY

Batch culture system	Mixed bacterial culture inoculated with single faecal sample from donor, growth in sealed vessel under anaerobic conditions, no additions (closed system). 24-72 hr culture. Usually performed in triplicate with faecal inoculae from up to four donors. Can compare multiple substrates and run multiple tests.
Biopsy	Small piece (few mm) of human tissue often extracted for examination to determine disease presence (usually a 'pinch biopsy' is performed).
Cross-feeding	Mechanism by which bacteria can grow, one bacterium utilising the growth substrates produced by another bacterium.
Cryoprotectant	Preservative added to cultures to maintain cell viability during freeze-drying.
Fermentor system	Mixed bacterial culture, e.g. inoculated with single faecal donor sample, growth in pH and temperature regulated continuous flow system, mostly under anaerobic conditions, with media continually replenished and volume maintained (open system). Ecosystem usually stable for 10-14 days. Labour intensive and limited to running sets of 3-6.
High throughput	Capacity for testing multiple variables/combinations simultaneously (>500).
Low throughput	Limited capacity for testing different combinations (<10).
Ileostomy	Surgically created loop of the small intestine directly towards the body outside. Body waste products can be excreted continuously through the loop. Procedure sometimes used temporarily or permanently following surgical interventions for bowel disease or colon / rectal cancer.
Inoculum/inoculae	Starting culture(s) used to introduce bacteria to a system. Can be a pure culture, mixed culture of known strains or mixed faecal inoculum from an individual donor. Usually performed with faecal inoculae from up to four donors.
***In silico* modelling**	Computer models used to simulate physiological processes. Can be used to predict how the microbiota will respond to specific dietary interventions, and how the resultant changes in the microbiota will impact human health. Also able to simulate what will happen if one parameter is changed. Only as good as the quality of laboratory information used to 'train' the model.
LBPs	Live Biotherapeutic Products. Biological medicinal products containing live micro-organisms as the active ingredient.
Luminal	The interior surface or contents of a hollow tube such as the intestine.
Microbiota profiling	Procedure used to determine the composition of the microbial community (microbiota). Often used to describe the bacterial profile rather than the complete microbial profile. Is most often on the genus, family or phylum level. Rarely on the species level.
Mucin layer	Gel-like (mucus) protective layer on the surface of epithelial cells. Mucin layer in the small intestine may differ from the large intestine.
Mucosal surface	The surface of the epithelial cell layer facing the inside, or lumen, of the intestine.
Regulatory bodies	EFSA – European Food Safety Authority EMA – European Medicines Agency FDA – Food and Drug Administration (U.S.A.)