



Nutritional enhancement of probiotics and prebiotics: Technology aspects on microbial viability, stability, functionality and on prebiotic function



PROTECH
(QLK1-2000-300042)

Project Coordinator

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Partners

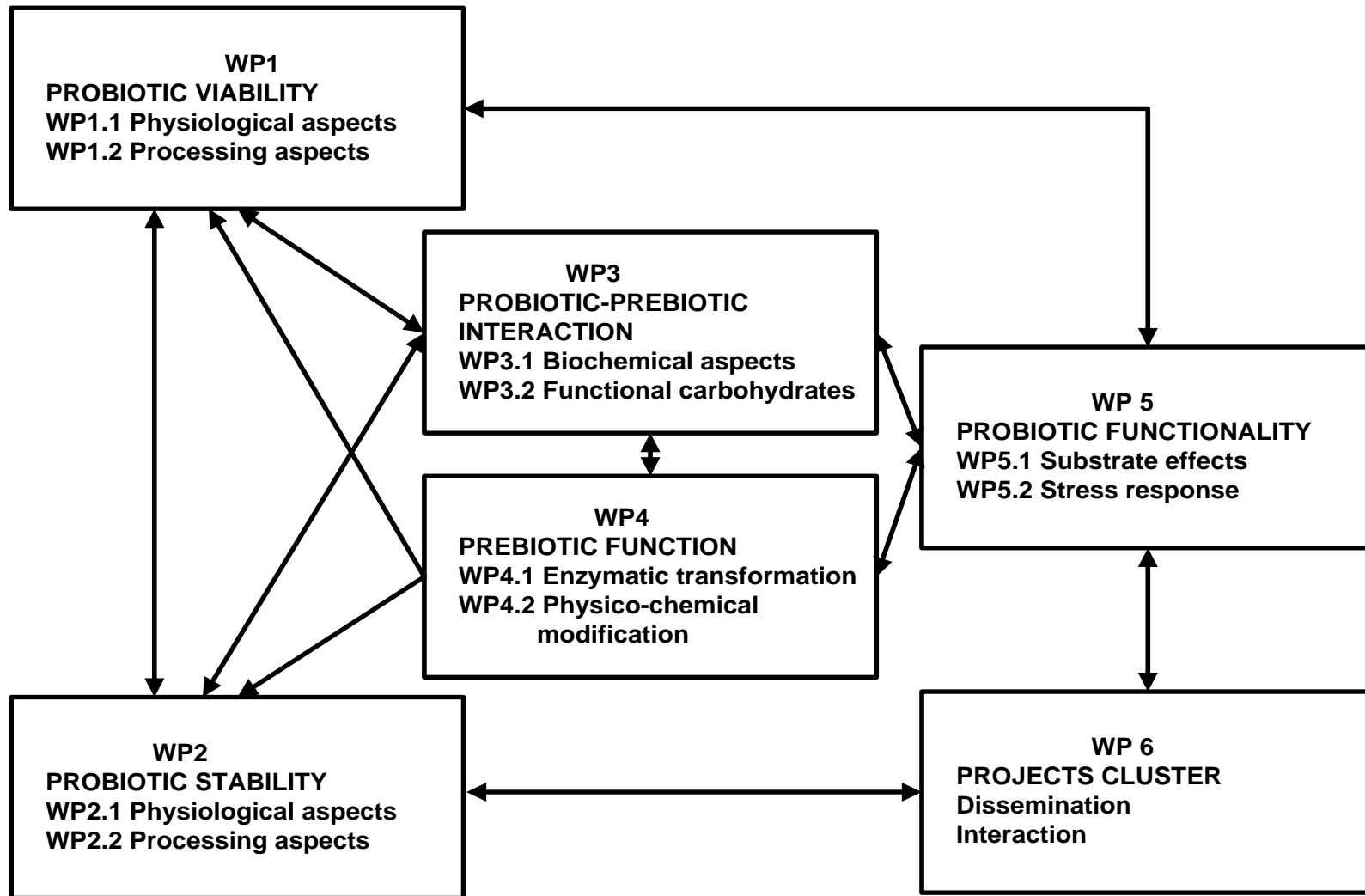
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-  VTT Biotechnology, Helsinki (M. Saarela)
-  Lund University, Lund (M. Nyman)
-  Wageningen University, Wageningen (F. Voragen)
-  TU Berlin Biotechnologie (U. Stahl)
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-  TINE Norwegian Dairy, Oslo (S.E. Birkeland)
-  University College Cork (G. Fitzgerald)
-  Dairy Research Centre, Moorepark, Cork (R.P. Ross)
-  ORAFIT, Brussels (R. Wouters)



PROEUHEALTH

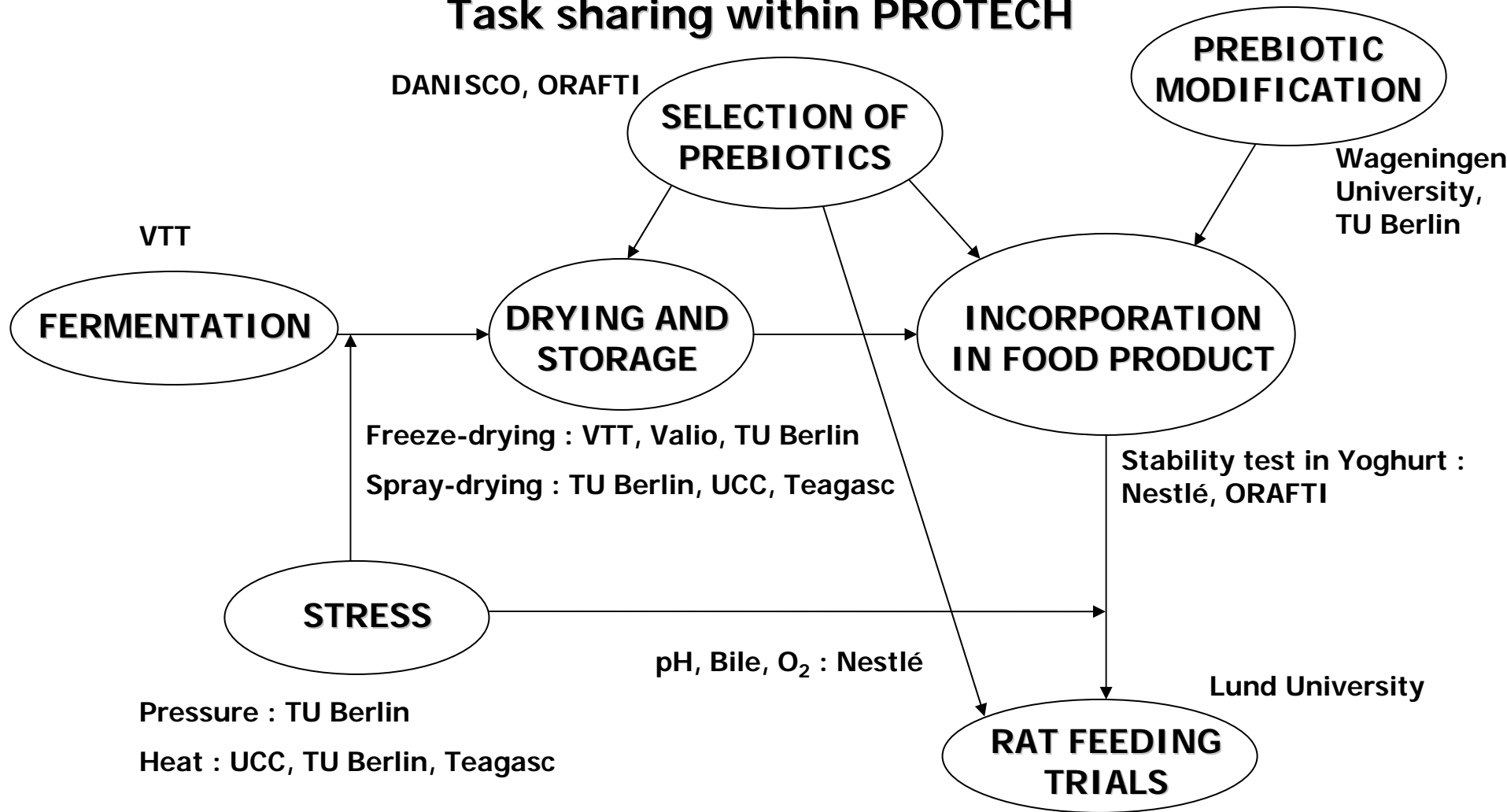


Translation of concepts in technical annex into...





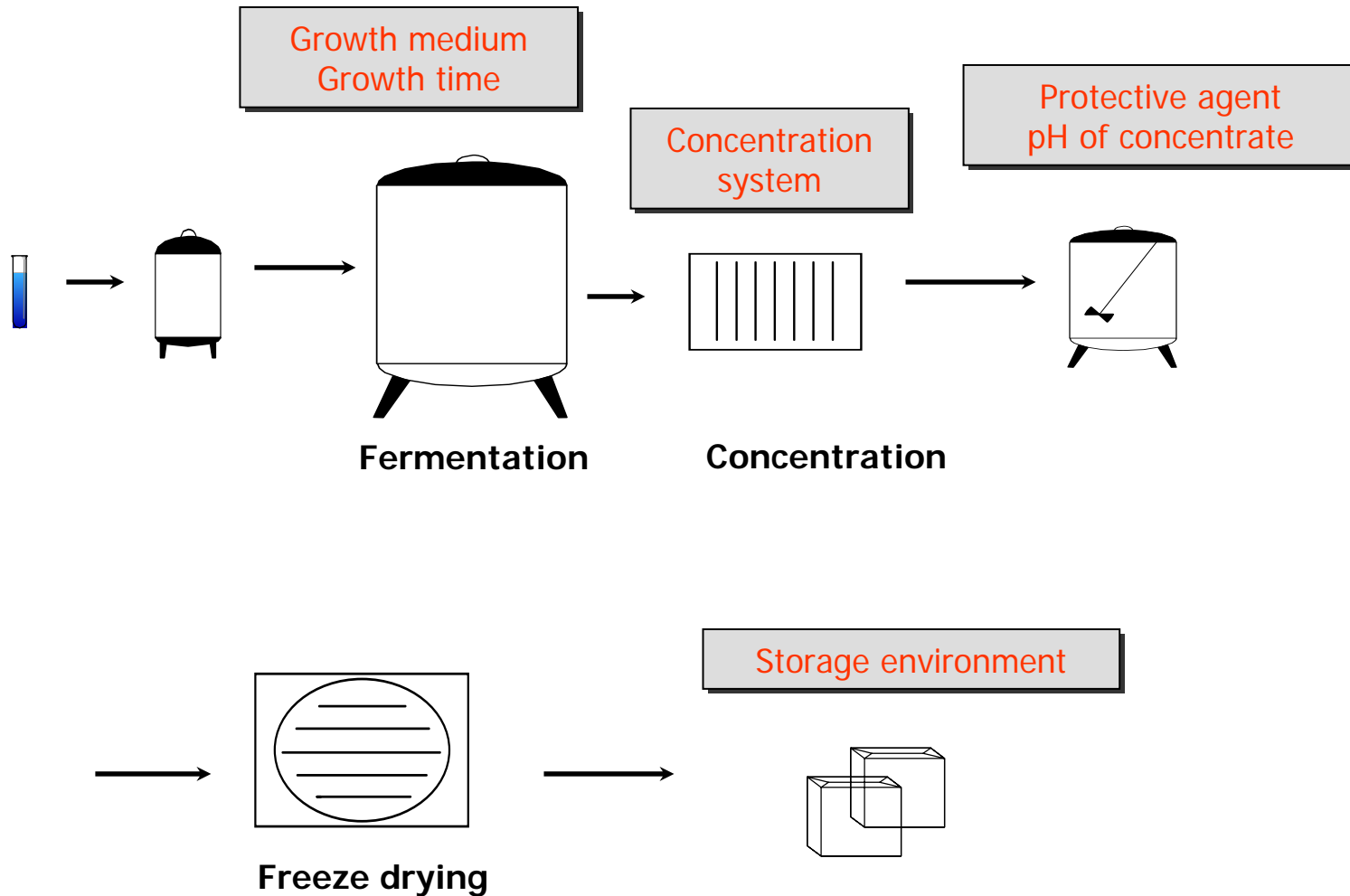
Task sharing within PROTECH



Ananta, E. , Birkeland, S.-E., Corcoran, B., Fitzgerald, G., Saarela, M. et al
Processing Effects on the Nutritional Advancement of Probiotics and Prebiotics
Microbial Ecology in Health and Disease 16 (2004) 113-124



FREEZE DRYING OF PROBIOTICS

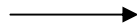




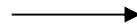
Optimized processes for the probiotic production

counts: 10^9 CFU/ml 10^{10} CFU/ml 10^{11} CFU/g

Fermentation



Concentration



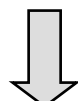
Cryoprotectants



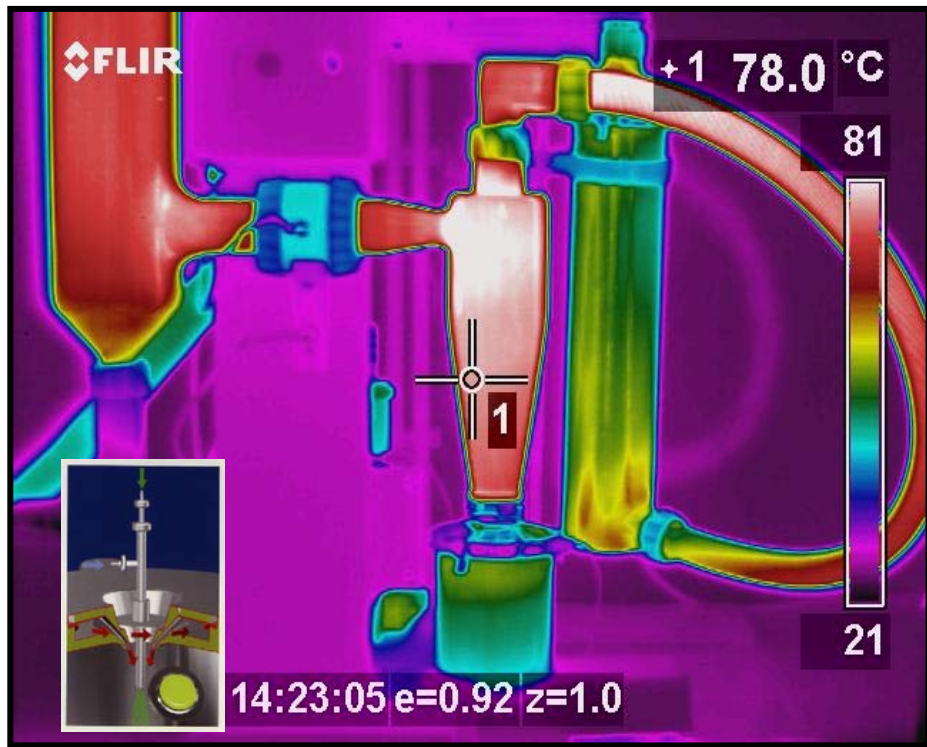
- Optimum for cells:
- Medium GeM
 - Time **15 h – 22 h**
 - Temperature 37°C
 - pH 5.8

- Adjustement of pH
- **pH5 – pH7**

- Protection with
- **milk based**
 - **milk free**
- cryoprotectant

- Optimal conditions
- 
- **survival**
 - **storage stability** at 37°C, 10°C, -20°C

SPRAY DRYING OF PROBIOTICS AND SYNBIOTICS

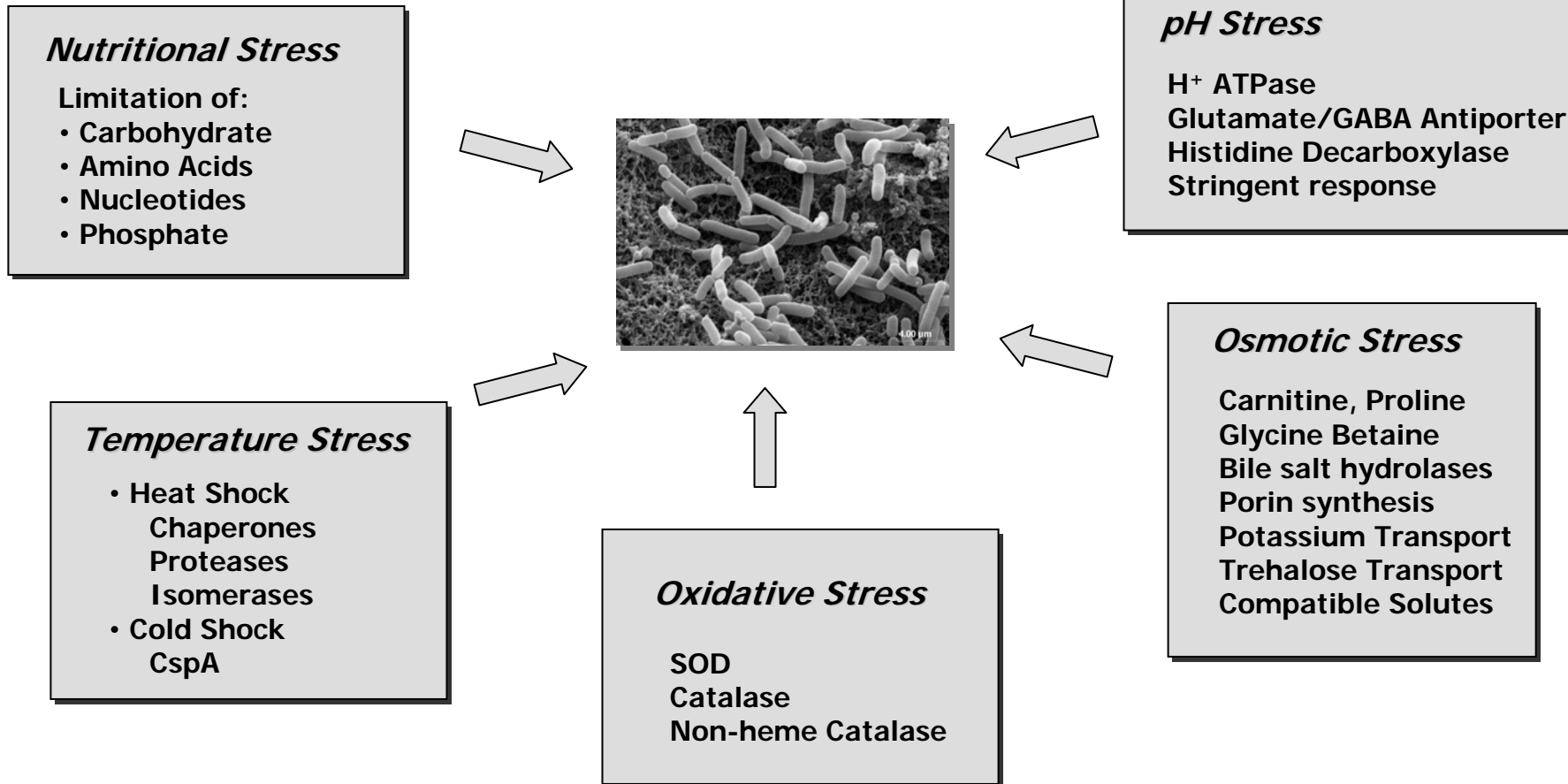


Type of drying equipment	Energy consumption (kj/kg water evaporated)
Membrane filtration (ultrafiltration and reverse osmosis)	50-150
Evaporator	
- one-step ("single-effect")	2600
- series ("double-effect")	1300
Spray dryer	
- one-step ("single effect")	6000
- series ("double effect")	4000
Drum dryer	5000
Tunnel dryer	4000
Freeze-dryer	100 000



TECHNOLOGICALLY INDUCED STRESS RESPONSE

Various stresses during production of LAB culture concentrates





VIABILITY & STABILITY OF PROBIOTICS IN FOOD MATRICES



ENZYMATIC MODIFICATION OF PREBIOTICS

- knowledge on the glycan-modifying machinery of *Bifidobacterium* is important to understand its utilisation of carbohydrates
- design of 'selective' prebiotics comes within reach

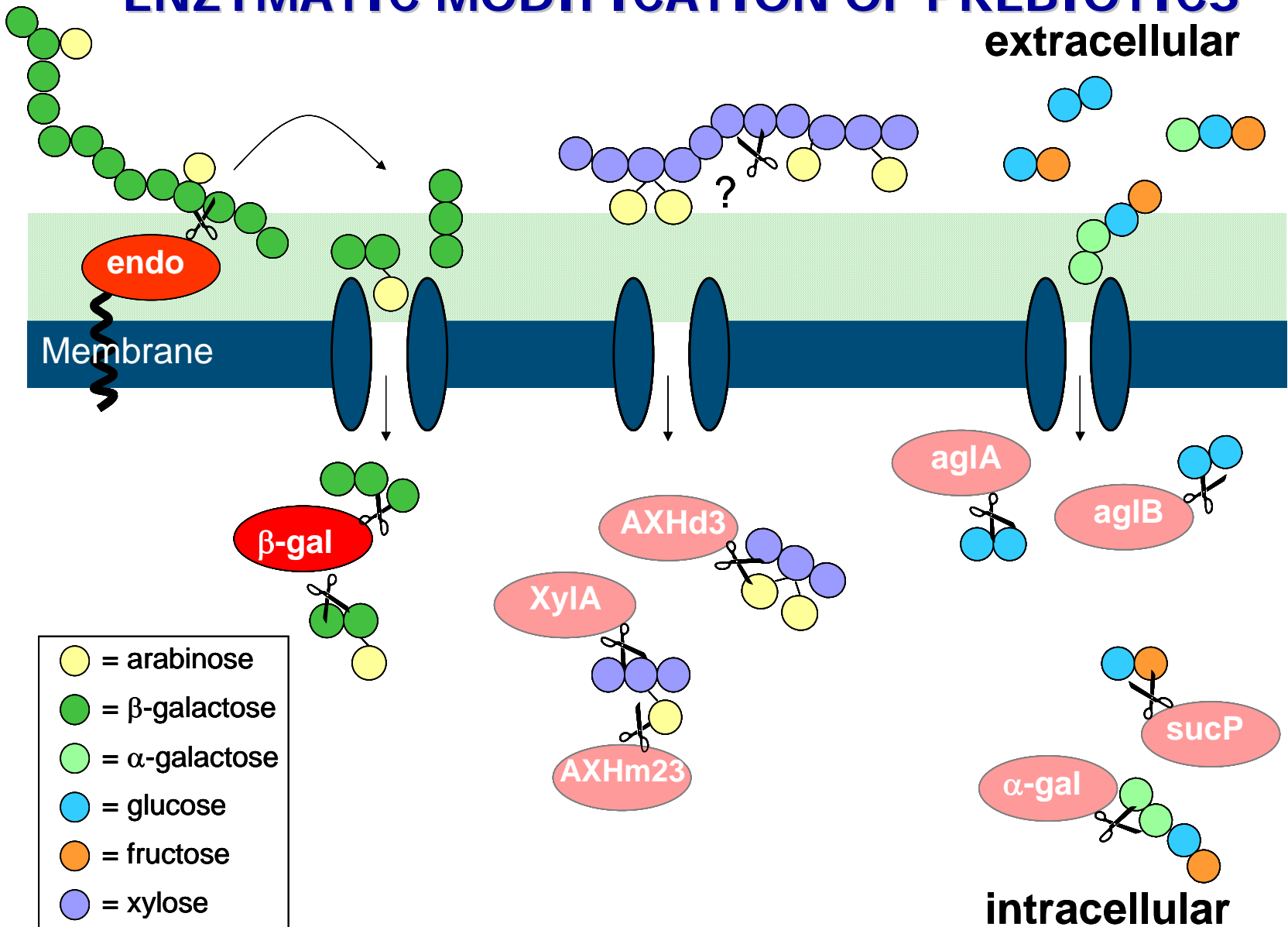


Special features of *Bifidobacterium* enzymes

- **α -galactosidase**: high transglycosylation activity
- **β -galactosidase II**: specific for β -1,4-linked galactosyl residues
- **endo-galactanase**: extracellular, membrane-bound enzyme releasing galactotriose from galactan
- **xylan-specific arabinofuranosidases**: complete removal of side-chains of cereal arabinoxylans

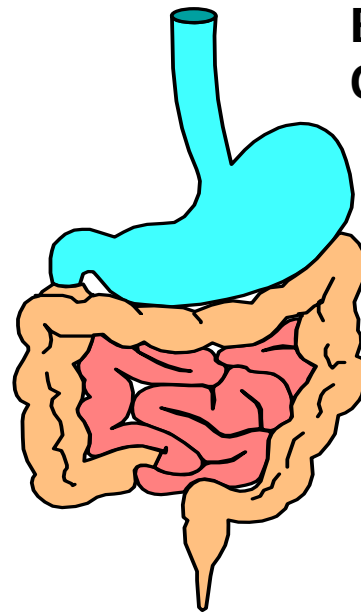
ENZYMATIC MODIFICATION OF PREBIOTICS

extracellular





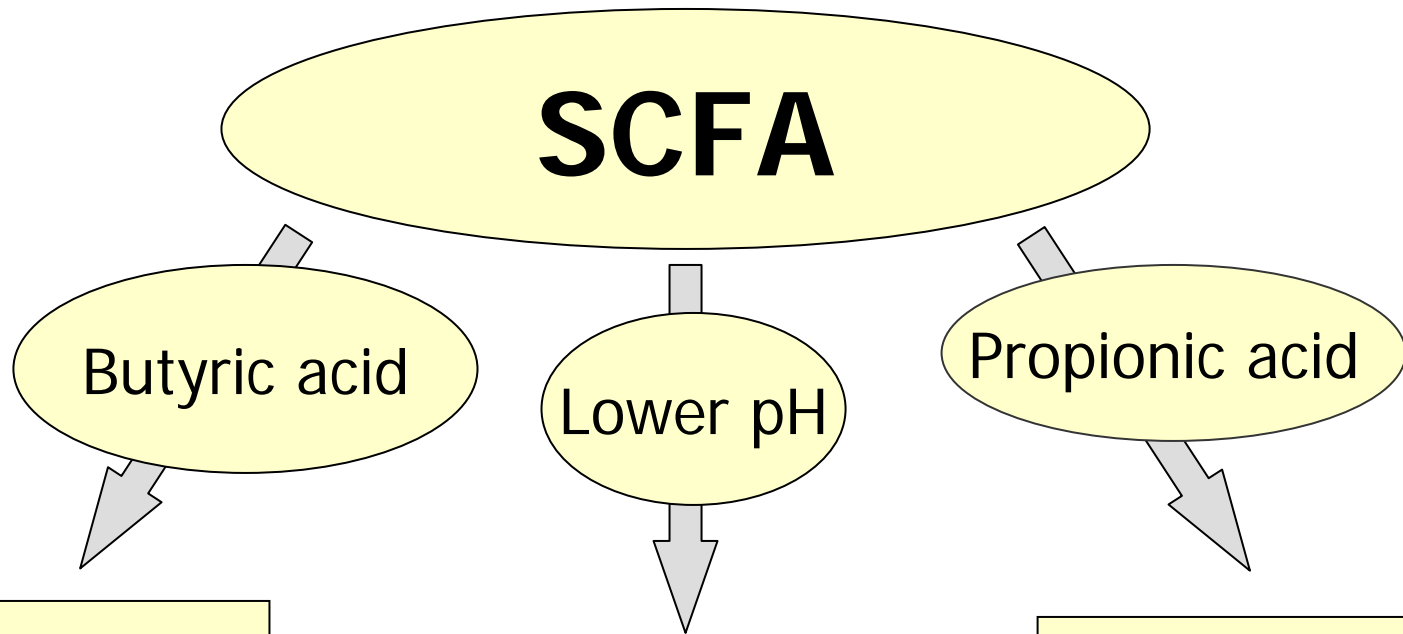
STABILITY & FUNCTIONALITY IN GI TRACT



Ensuring good
GI tract survival

Rat feeding trials -
SCFA pattern





- The most important substrate for the colonic mucosa
- Increased resistance towards injuries
- Decreased risk for colonic diseases

- Mineral absorption ↑
- Blood flow through the colonic mucosa ↑
- Mobility of the colon ↑
- Secondary bile salts ↓

- Affects glucose- and lipid-metabolism positive
- Anti-inflammatory effect



SUMMARY OF GENERAL ACHIEVEMENTS

FERMENTATION

Development of milk-free general and specific media for probiotics as an alternative growth media to standard broth (MRS)

Evaluation of technological suitability of probiotic strains

FREEZE DRYING

Identification of critical factors for high freeze drying survival (growth time, pH, cryoprotectants, storage temperature)

Establishing optimized freeze drying protocols as well as test procedures (acid- and bile-tolerance, storage stability)

PROBIOTIC STABILITY IN FOOD SYSTEMS

Identification of critical environmental factors governing survival and vitality (pH, storage temperature, type of yoghurt strains, prebiotics)



SUMMARY OF GENERAL ACHIEVEMENTS

STRESS RESPONSE

Identification of stress induced proteins using proteomic approach

Evaluation of the importance of cross-adaptive response (pressure, salt, heat, etc.) in improving tolerance against lethal effect of heat, drying and bile

MODIFICATION OF PREBIOTICS

Cloning, purification and characterization of bifidobacterial glycolytic enzymes (β -gal II, endo-galactanase)

Demonstration of the possibility to perform modification on polysaccharides using high hydrostatic pressure

ROLE OF PRE- AND PROBIOTICS IN DIET

Modulation in SCFA pattern upon feeding rats with prebiotics and/or probiotics



ACHIEVEMENTS BEYOND TECHNICAL ANNEX

SPRAY DRYING

Identification of critical process parameters (temperature, drying carrier, etc.) as well as optimal processing regimes

Possibility of incorporating prebiotics in drying carrier

Assessment of storage stability and factors governing viability retention (storage temperature, atmosphere, glassy state, etc.)

Establishing a flow cytometric protocol to investigate drying induced cellular damages