Application of flow cytometry for enumerating individual bacterial cultures from a mixed culture system

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Abstract

Cultured dairy products are often made with more than one microbial culture. Yoghurt requires the cultivation of several bacterial species for its production and the level of each is important for different reasons. Differential plate count methods to enumerate the separate species in yoghurt are not ideal because many of the bacteria used have similar growth profiles and plate counts take several days to produce a result. A fast specific method for enumerating each culture would be beneficial because quick results would enable tighter control of processing or experimental conditions and the ability to track individual species amongst a background of similar bacteria. Flow cytometry combined with fluorescent *in-situ* hybridisation (FLOW-FISH) was investigated as a potential solution and successful enumeration was achieved within 1 day for a yoghurt microorganism, *Streptococcus thermophilus* (ST55), grown in M17 medium. This method may be improved to increase the signal-to-noise ratio and to reduce the assay time. The chemical propidium monoazide enabled a closer match to plate counts for flow cytometry results using a total viable count assay and may be useful combined with the FLOW-FISH assay for removing non-viable or viable, but non-culturable, cells from the results. An enzyme and/or detergent pre-treatment may achieve successful FLOW-FISH enumeration of cells grown in reconstituted skim milk – a similar matrix to yoghurt.

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Abbreviations

AC	Aerobic count
BFM	Agar growth medium for *Bifidobacteria* sp.
CFU	Colony forming units
Cy3 and Cy5	Cyanine dyes
DAPI	4',6-Diamidino-2-Phenylindole
DGGE	Denaturing gradient gel electrophoresis
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOPE	Double labeling of oligonucleotide probes
EMA	Ethidium monoazide
FCM	Flow cytometry
FISH	Fluorescent in situ hybridization
FITC	Fluorescein isothiocyanate
FL1 or FL2	Fluorescence level 1 or 2
FLOW-FISH	FISH combined with flow cytometry
LED	Light emitting diode
M17	Agar or broth growth medium for *Streptococcus thermophilus*
MRS	deMan-Rogosa Sharpe growth medium for *Lactobacillus* sp.
N	Sample size
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PI	Propidium iodide
PMA	Propidium monoazide
PMA-FLOW-FISH	PMA treatment of cells combined with a FLOW-FISH assay
PNA	Peptide nucleic acid
PMT	Photomultiplier tube
RB	Raffinose Bifidobacterium: a selective agar medium for *Bifidobacteria*
RCA	Reinforced Clostridial agar
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
RSM	Reconstituted skim milk medium
RT-PCR	Real time-polymerase chain reaction
SD	Standard deviation

SEM	Standard error of the mean
Sth	*Streptococcus thermophilus*
ST55	*Streptococcus thermophilus* strain number
SYL	Agar growth medium that allows the growth of both *Streptococcus thermophilus* and *Lactobacillus bulgaricus*
SYTO® 9	Molecular Probes fluorescent stain
T_m	Melting temperature of a duplex DNA-DNA or DNA-RNA molecule
TPPYPB	Tryptone-proteose,peptone-yeast extract with Prussian blue agar growth medium
TVC	Total viable count
UHT	Ultra-heat treated
VBNC	Viable, but non-culturable