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The Microflora of Raw Milk and the Impact of Milk on their Survival at Low pH

A Thesis Presented in Partial Fulfilment of the Requirements for the Degree of
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Abstract

Milk is an excellent food source as it contains a plentiful supply of nutrients and minerals. Although normally consumed after pasteurisation, there is growing evidence that raw milk provides health benefits beyond nutrition alone. Epidemiological studies of children have shown that those who regularly consume raw milk appear to have a lower incidence of asthma and non-specific allergy than those who consume processed commercial milk. The gastrointestinal tract is a key location for immune development as interaction with microflora can occur at the mucosal surface. Milk may have a role to play in the early stages of this process either due to the microbes it harbours or to the physical and chemical properties of milk itself.

The aim of this study was to identify bacterial isolates unique to raw milk, that would not survive pasteurisation; and to determine whether milk allowed for a greater survival of these bacteria during ingestion. Bacterial isolates were cultured from either raw or pasteurised milk and tested for their ability to survive pasteurisation. A subset of thermosensitive isolates were identified for further analysis representing those species likely to be present in raw milk but not processed. This thermosensitive subset was challenged for their ability to tolerate acid conditions (pH 2.5) both in the presence and absence of milk to determine the likelihood of their survival during ingestion. A high throughput acid tolerance test was developed to screen raw milk bacteria for acid tolerance. Data supports the hypothesis that milk significantly increased the survival of raw milk bacteria exposed to pH 2.5 and that specific components found specifically in milk were, at least in part responsible for this effect. In conclusion, a unique subset of bacteria found only in raw bovine milk, and not in processed milk, has been identified that when ingested with milk are able to come through an acid challenge not dissimilar to that of the stomach and survive. This opens the possibility that these bacteria present in raw milk are able to enter the lower GI tract and interact with the immune system via Peyers patches.
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Abbreviations

aOR  adjusted Odds Ratio
APC  Antigen Presenting Cell
APT  All Purpose Tween
CFU  Colony Forming Units
CI   Confidence Interval
CM   Casein Micelle
DC   Dendritic Cell
DGGE Denaturing Gradient Gel Electrophoresis
EDTA Ethylenediaminetetraacetic acid
GAD  glutamic acid decarboxylate
GI   Gastrointestinal
GLMM Generalised Linear Mixed Model
Ig   immunoglobulin
IL   Interleukin
IFN-α Interferon-alpha
LB   Luria Bertani
LSD  Least Significant Difference
M    Molar (mol / L)
M-PCA Milk-Plate Count Agar
mM   milli-Molar
MRD  Maximum Recovery Diluent
OR   Odds Ratio
PCA  Plate Count Agar
r16S ribosomal 16S
SD   Standard Deviation
SSCP Single Strand Conformational Polymorphism
T_H1 T-Helper type 1
T_H2 T-Helper type 2
TSA  Tryptic Soy Agar
TSB  Tryptic Soy Broth
UHT  Ultra-high Temperature
WPC  Whey Protein Concentrate