Fermented Products and their Potential Influences on Health and Disease

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DISCLOSURE

• The information presented here is my personal opinion and does not represent National Cancer Institute official position.

• I have no actual or potential conflict of interest in relation to this presentation.
Historical Consumption of Fermented Foods

Humans consume fermented food since ancient times as a result of the natural fermentation process which precedes human history.

- Controlled fermentation was used historically since 8000 BC to produce alcoholic beverage from fruits, rice, and honey.
- Fermentation processes involve the use of
  - yeast to produce alcohol from carbohydrates
  - bacteria such as lactobacillus, for producing yogurt, kefir, cheese and sauerkraut.

- The science of fermentation is known as zymology.
Historical Consumption of Fermented Dairy Products

- Fermented milk products have been produced and consumed since around 10,000 BC.
- In the beginning of the 20th century, the Russian Nobel prize winner Élie Metchnikoff observed a high life expectancy in Bulgarian persons who ate large amounts of yogurt.
- Milk products/cultured dairy products, are mostly produced using lactic acid bacteria such as Lactobacillus, Lactococcus, and Leuconostoc.
- The fermentation process
  - increases the shelf-life of the product
  - enhances the taste
  - improves the digestibility of milk.
  - health benefits?
Probiotics, Prebiotics and Synbiotics

• **The Probiotics** definition as originally framed by WHO/FAO is broadly accepted and as "live microorganisms which, when administered in adequate amounts confer a health benefit on the host.

• **Prebiotics** are “non-viable food components that confer a health benefit on the host associated with modulation of the microbiota”. By definition, constitute a selective substrate for one or a limited number of beneficial bacteria and are able to alter the colonic microbiota in favor of a healthier composition.

• **Synbiotics**: the combination of probiotics and prebiotics.
## Fermented Dairy Produced by Lactic Acid Bacteria

<table>
<thead>
<tr>
<th>Product</th>
<th>Alternative names</th>
<th>Typical milkfat content</th>
<th>Typical shelf life at 4°C</th>
<th>Fermentation agent</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese</td>
<td></td>
<td>1–75%</td>
<td>varies</td>
<td>a variety of bacteria and/or mold</td>
<td>Any number of solid fermented milk products.</td>
</tr>
<tr>
<td>Cultured buttermilk</td>
<td></td>
<td>1–2%</td>
<td>10 days[1]</td>
<td>Lactococcus lactis*[4][1], Lactococcus lactis subsp. cremoris, Lactococcus lactis biovar. diacetylactis and Leuconostoc mesenteroides subsp. cremoris[1][2]</td>
<td>Mesophilic fermented pasteurized milk</td>
</tr>
<tr>
<td>Acidophilus milk</td>
<td>acidophilus milk</td>
<td>0.5–2%</td>
<td>2 weeks[1]</td>
<td>Lactobacillus acidophilus[1][3]</td>
<td>Thermophilic fermented milk, often lowfat (2%, 1.5%) or nonfat (0.5%), cultured with Lactobacillus acidophilus</td>
</tr>
<tr>
<td>Crème fraîche</td>
<td>creme fraiche</td>
<td>30–40%</td>
<td>10 days[1]</td>
<td>naturally occurring lactic acid bacteria in cream</td>
<td>Mesophilic fermented cream, originally from France; higher-fat variant of sour cream</td>
</tr>
<tr>
<td>Filmjölk</td>
<td>fil</td>
<td>0.1–4.5%</td>
<td>10–14 days[1]</td>
<td>Lactococcus lactis* and Leuconostoc[4][5]</td>
<td>Mesophilic fermented milk, originally from Scandinavia</td>
</tr>
<tr>
<td>Viili</td>
<td>filbunke</td>
<td>0.1–3.5%</td>
<td>14 days[1]</td>
<td>Lactococcus lactis subsp. cremoris, Lactococcus lactis* biovar. diacetylactis, Leuconostoc mesenteroides subsp. cremoris and Geotrichum candidum[6]</td>
<td>Mesophilic fermented milk that may or may not contain fungus on the surface; originally from Sweden; a Finnish specialty[6]</td>
</tr>
<tr>
<td>Kefir</td>
<td>kephir, kewra,</td>
<td>0–4%</td>
<td>10–14 days[1]</td>
<td>Kefir grains, a mixture of bacteria and yeasts</td>
<td>A fermented beverage, originally from the Caucasus region, made with kefir grains; can be made with any sugary liquid, such as milk from mammals, soy</td>
</tr>
</tbody>
</table>

# Lactic Acid Bacterial Strains and Their Anticancer Functions

<table>
<thead>
<tr>
<th>Prevention</th>
<th>LAB strains</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis</td>
<td><em>Lactobacillus acidophilus</em></td>
<td>Anti-cancer cell growth and differentiation, Direct induction of Beclin-1 and GRP78, Proliferation (Cox-2, cyclin D1) and cell survival (Bcl-2, Bcl-xL)</td>
</tr>
<tr>
<td></td>
<td><em>L. reuteri</em></td>
<td>Enhances MAPK activities including c-Jun N-terminal kinase and p38 MAPK, Induce Beclin-1 and GRP78, as well as indirectly through the induction of Bcl-2 and Bak</td>
</tr>
<tr>
<td>Antioxidant DNA damage</td>
<td><em>L. acidophilus</em> and <em>L. rhamnosus</em></td>
<td>5-fluorouracil apoptosis induction</td>
</tr>
<tr>
<td></td>
<td><em>Bifidobacterium longum</em> and <em>L. acidophilus</em></td>
<td>Antioxidative activity, inhibiting linoleic acid peroxidation</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus thermophilus</em></td>
<td>Releasing ROS protective factors</td>
</tr>
<tr>
<td></td>
<td><em>L. acidophilus</em></td>
<td>Stimulates DCs to produce inflammatory cytokines IL-12 and regulatory IL-10, Induces IL-10 in DCs, down-regulates IL-12 levels</td>
</tr>
<tr>
<td>Immune response improvement</td>
<td>LTA-deficient <em>L. acidophilus</em></td>
<td>Increases densities of effector Foxp3⁺RORγT⁺ Tregs, Enhance the total numbers of T cells, NK cells, MHC class II+ cells, and CD4-CD8+ T cells</td>
</tr>
<tr>
<td></td>
<td><em>L. acidophilus</em>, <em>L. casei</em> and <em>B. longum</em></td>
<td>Induces cytokines, such as IFN-γ, interleukin-β (IL-1β) and TNF-α</td>
</tr>
<tr>
<td></td>
<td><em>L. casei Shirotai (LcS)</em></td>
<td>Increases the production of TNF-α</td>
</tr>
<tr>
<td></td>
<td><em>B. adolescentis</em></td>
<td>Enhances the expression of tumor suppressor genes</td>
</tr>
<tr>
<td>Epigenetics</td>
<td>LTA-deficient <em>L. acidophilus</em></td>
<td>Adhere to colon cancer cells and trigger bioproduction of SCFA</td>
</tr>
<tr>
<td></td>
<td><em>Pediococcus pentosaceus</em> FP3</td>
<td></td>
</tr>
<tr>
<td>New anticancerfunction</td>
<td><em>L. salivarius</em> FP25</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>L. salivarius</em> FP35</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Enterococcus faecium</em> FP51</td>
<td></td>
</tr>
</tbody>
</table>

LAB: Lactic acid bacteria; LTA: Lipoteichoic acid; SCFA: Short-chain fatty acid; DC: Dendritic cell; NK: Natural killer; IL: Interleukin; TNF: Tumor necrosis factor.
Probiotic Flora and Microbiome – Defining Health and Disease

Sanders, ME et al Gut Microbes 2011; 2:3 127
Mechanisms in Probiotic Interaction with Intestinal Epithelium

1. Competitive exclusion of pathogenic bacteria adhesion

2. Stimulation of a protective humoral immune response (production of IgA, IgG, IgM);

3. Antimicrobial activities, e.g. by decreasing pH, increasing bacteriocin or H₂O₂;

4. Enhancing mucosal barrier integrity;

5. Release of polyamines (e.g. spermine, spermidine);

6. Inhibition of the inflammatory response (e.g. NF-κB and MAP Kinases).

Girardin M, Dig Dis 2011;29:574
Potential Mechanisms of Action of Lactic Acid Bacteria via Extrinsec and Intrinsec Pathways of Apopotosis

Intrinsic pathway:
- LAB possibly enhances the apoptotic activity of 5-FU and it may induce the activation of autophagic cell death.

Extrinsic pathway:
- Engages FAS/tumor necrosis factor receptors to induce caspase related pathway.

Li Zhong et. al World J Gastroenterol.2014
LAB may induce immune responses through two main pathways:

- **Inflammation**: involves lipotheicoic acid (LTA) and interleukin release;

- **Anti-tumor immuno-effects**: stimulate T cells, dendritic cells and natural killer cells.
Effects of growth inhibition by *B. adolescentis* SPM0212 on colon cancer cell lines (Caco-2, HT-29 and SW480).

- The cells were treated with *B. adolescentis*
- The absorbance was measured using an ELISA reader at 490 nm.
- The quantitative data were presented as means ± SD of three independent experiments.
- Control versus treatment groups, *p < 0.05; **p < 0.01.

Do Kyung Lee et al. BMC Cancer. 2008;
Fermentation supernatants of *Lactobacillus delbrueckii* inhibit growth of human colon cancer cells and induces apoptosis

Effect of LBF solution on the proliferation of colon cancer SW620 cells.

The SW620 cells were treated with various concentrations of LBF solution for 24 h. Growth is expressed as relative to untreated control cells. Multiple comparison test was used for statistical analysis. **P<0.01 and ***P<0.001, vs. untreated control cells. LBF, *Lactobacillus delbrueckii* fermentation
Effect of *L. Acidophilus* and *L. Casei* mix in the presence of 5-fluorouracil (5-FU) on LS513 cell apoptosis

Live Bacteria

Microwave-inactivated
Oral inoculation of probiotics *Lactobacillus acidophilus* NCFM suppresses tumour growth both in segmental orthotopic colon cancer and extra-intestinal tissue.

*Lactobacillus acidophilus* NCFM (*La*) pre-inoculation decreased subcutaneous CT-26 tumour growth.

(a) Tumour volumes were determined for CT-26 (*), CT-26+*La* (*) and CT-26+*Ec* () mice.

(b) Representative CT-26, CT-26+*La* and CT-26+*Ec* tumours are shown. CT-26 alone, mice were implanted with CT-26 cells; CT-26+*La*, mice were pre-inoculated with *La* $1 \times 10^8$ colony-forming units (cfu)/mouse per d; CT-26+*Ec*, mice were pre-inoculated with *Escherichia coli* K12 $1 \times 10^8$ cfu/mouse.

Preventive effects of probiotic bacteria Lactobacillus plantarum and dietary fiber in rat mammary carcinogenesis.

The PRO-PRE combination:

- lowered tumor incidence (by 17%) lowered tumor volume (by 37%).
- decline tumor frequency per group (by 47%, 47% and 49%, respectively).
- reduce the frequency per animal (by 44%, 41% and 39%, respectively).

IC: Invasive carcinomas; ISC: in situ carcinomas; B: benign lesions

Clinical trial: the effects of a fermented milk containing three probiotic bacteria in patients with irritable bowel syndrome – a randomized, double-blind, controlled study Sweden

The overall GI symptom severity, assessed by IBS-SSI.

FU = follow-up 8 weeks after the end of the treatment period.

Mean, s.d.

* $P < 0.05$

** $P < 0.01$

vs. baseline.
CONCLUSION: These findings suggest that a high intake of cultured milk may lower the risk of developing bladder cancer.
Models are adjusted for age, smoking status, number of cigarettes smoked, smoking duration, and intakes of vegetables, fruits, meat, beverages, energy, and fat.

Hazard ratios (solid line) and 95% confidence intervals (dashed lines) for bladder cancer in men (A) and women (B)
Consumption of Dairy Products and Colorectal Cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC).

Yoghurt

- Yoghurt intake was significantly inversely related to colorectal cancer risk in categorical models (≥109 g/day vs. non-consumers, HR 0.90, 95% CI: 0.81–0.99; \( P \)-trend =0.043). The inverse association was restricted to the colon and not observed for tumours in the rectum, although the difference was not statistically significant (\( P \) Heterogeneity =0.79).

Cheese

- Cheese consumption was inversely associated with colorectal cancer in the categorical model. The association was significant for colon (≥56 g/day vs. <5 g/day HR, 0.83, 95% CI: 0.71–0.97; \( P \)-trend =0.047) but not rectal cancer, although this difference was not significant (\( P \) Heterogeneity =0.39).

- Murphy N et. al PLoS One 2013 Sep
Milk Consumption and the Risk of Prostate Cancer

• Probable association between milk intake and lower risk of colorectal cancer

• Probable association between milk intake and increased risk of prostate cancer

• Limited association between milk intake and lower risk of bladder cancer
Conclusions and Future Directions

- Fermented dairy products has shown some benefits in preventing certain types of cancer in cell cultures and animal studies under specific conditions.
- Few cohort and clinical trial shown some benefits for colon and bladder cancer.
- More studies need to establish:
  - the subgroups who would benefit the most and the ones at risk.
  - the most effective synbiotic products,
  - the amount, frequency and duration of consumption to obtain optimal response.
  - other diseases that could be prevented/delayed by the consumption of fermented dairy products.
Thank you for your attention