Indigenous Probiotics and Immunological Effects

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TRADITIONAL WAY OF DADIH MAKING

Fresh raw unheated buffalo milk, poured into bamboo tubes

The inspiration
Dadih, Indonesian traditional fermented raw buffalo milk of West Sumatra

*Lactobacillus plantarum IS-10506, Enterococcus faecium IS-27526*, isolated from Dadih have probiotic properties
Probiotics: Concept & Definition

Elie Metchnikoff (1845-1916)
“The prolongation of life - Optimistic studies”

- Longevity of Caucasians relate to yogurt consumption
- Most gut microbes have detrimental effects
- Replacement of the gut microflora by yogurt microbes is beneficial

“Transformation of the wild population of the intestine into a cultures population ... the pathological symptoms may be removed from old age, and ... the duration of life of man may be considerably increased”

Probiotics are live microorganisms, which when consumed in adequate amounts, confer a health effect on the host

FAO/WHO, 2001

Probiotic effects are strain specific

Probiotics in Nutrition
- Anti-infections (gut balance)
- Immune stimulation (immune defenses)
- Allergy prevention or treatment
- Modulation of inflammation

Lactobacilli  Bifidobacteria
YOUR BODY IS A PLANET

Of the 100 trillion cells inside each one of us, only 10 percent are actually human. The rest belong to aliens: bacteria, fungi, and other microbes.

BY JOSIE CLAUSIUSZ

We may not realize it, but each one of us is a walking ecosystem. Microscopic, right-linged Drosophila flies wander hand down inside the follicles of the eyelashes, festillinating unaided skin cells. Microscopic yeasts live on the tongue, teeth, and skin, and in the intestine. Dormant viruses like herpes simplex may lie dormant for years inside nerve cells. Perhaps strangest of all are the self-replicating, visko-like pieces of DNA that infected ancient humans and still make up about 8 percent of our genome.

Most of the time we share our bodies harmoniously with the 50 billion or so microbes. But sometimes the arrangement turns contentious, as when blood-sucking bedbugs, fleas, and lice invade, or when herpes simplex or human papillomavirus causes surface membranes to split in nasty pustules or warts. Just taking antibiotics may disturb the ecosystem in our gut by killing not only disease-causing organisms but also good bacteria, like Lactobacillus acidophilus. Living with microbes demands a biological balancing act. For the most part, we are biologically oblivious to the microscopic life we carry around with us. Considering what those organisms look like, that may be a good thing.

3. FRANKIE & BACTERIOIDES

At least 1000 species of bacteria, weighing about 3.2 pounds, live inside the human gut. Traditionally, you remove one of these phyla, the Firmicutes and the Bacteroidetes. They break down carbohydrates and make essential nutrients like vitamin K and B12. They also ensure healthy bacteria. In Cynthia Sears at Johns Hopkins Center for Global Health says, "If you lose a force of members, the bad bugs are beat out by the good bugs."

4. HUMAN PAPILLOMAVIRUS

More than 100 different human papillomaviruses (HPV) can infect the skin, and there are dozens from the common wart to plantar and nail warts. At least 1% of women are sexually transmitted, and the CDC estimates that at least 50 percent sexually active men and women will be infected with genital HPV at some point. Of greater concern are HPV types 16 and 18, which can cause cancers of the cervix, penis, vulva, anus, and neck. The new vaccine Gardasil protects against the cancers caused by both HPV types.

8. SHINGLES

Once you have had chickenpox, the virus, called varicella-zoster, stays inside you forever, lying dormant in nerves near the spinal cord. It can become active and cause a painful, itchy rash called shingles, which can cause a significant increase in skin cancers among the elderly.

Glausiusz J. Discover June 2007
What makes a human?

• Human metabolic features- combo of human and microbial traits
• Microbiota- microrganisms that live inside and on humans
• Microbiome* - the genomes of the microbial symbionts

*totality of microbiota and their genetic elements (genomes), and environmental interactions. (Joshua Lederberg), influence human physiology. Entire microbiome only weighs about 200 grams (7.1 oz). Coyle, MD, Walter J.

"The Human Microbiome: The Undiscovered Country"
• Humans are 90% bacteria
  – Cell counts
  – 99+% by gene diversity
• Communication and cross-feeding
• Integrates with systems
• There is a ‘normal microbiota’
• Microbiota can ‘mis-provide’ functions
  – dysbiosis*

* Sartor RB; and Sokol et al 2008 PNAS 105
Screening and Selection of Potential Probiotic Strains
Adhesion Properties of Indigenous Dadih Lactic Acid Bacteria on Human Intestinal Mucosal Surface

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ABSTRACT: Dadih is Indonesian traditional fermented buffalo milk believed by the natives to have beneficial effects on human health. This may be due to the probiotic properties possessed by the lactic acid bacteria (LAB) involved in its fermentation process. It was discovered that ten strains of dadih lactic isolates possessed some probiotic properties in vitro. In this study, the adhesion properties of dadih LAB, in comparison with documented probiotic strains, were investigated in vitro by using mucin extracted from human faeces and Caco-2 cells as the models for human intestinal mucosal surface and intestinal cells respectively. The adhesion results showed the distinction of Lactobacillus reuteri IS-27560 in adhering to both mucus layer and Caco-2 cells. The competition assay for adhesion to the mucus layer between dadih LAB and selected pathogens indicated the competence of Lactococcus lactis IS-16183 and Lactobacillus rhamnosus IS-7257 in significantly inhibiting the adhesion of Escherichia coli O157:H7. Accordingly, these two strains may be potential candidates for use as probiotic strains. Overall, the adhesion properties of all dadih LAB strains were relatively comparable to that of Lactobacillus casei Shirota and Lactobacillus rhamnosus GG, the documented probiotic strains. (Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 5 : 751-755)

Key Words: Dadih, Lactic Acid Bacteria, Adhesion, Probiotics, Mucin, Caco-2 Cells
DHARMAWAN ET AL.

LAB adhered (cfu/well)

LAB

Indigenous Dadih Lactic Acid Bacteria: Cell-Surface Properties and Interactions with Pathogens

M. Carmen Collado, Ingrid Surono, Jussi Meri luoto, and Seppo Salminen

ABSTRACT: Cell surface properties of dadih lactic acid bacteria strains were studied for adhesion to hydrocarbons (BATH) and aggregation abilities. Autoaggregation correlates with adhesion, which is a prerequisite for colonization and infection of the gastrointestinal tract by many pathogens, whereas coaggregation has been related to the ability to interact closely with pathogens. The results demonstrated significant differences in cell surface properties among the tested natural lactic acid bacteria food strains. Hydrophobicity increased when the cells were heat inactivated. All strains showed aggregation abilities with the pathogen strains tested, but the coaggregation properties were strain-specific. Our results indicate that the ability to autoaggregate, together with cell surface hydrophobicity and coaggregation abilities with pathogen strains, can be used for preliminary screening in order to identify potentially probiotic bacteria suitable for human or animal use. This study suggests the importance of identifying and characterizing bacterial cell-wall properties to understand their role in adhesion to hydrocarbons, autoaggregation and relation to coaggregation mechanisms, and also the relevance to future probiotic food development from natural strains.

Keywords: adhesion, coaggregation, dadih, pathogens, probiotics

Figure 1 – Autoaggregation abilities (%) of the probiotic bacteria isolated from dadih as a function of adhesion of xylene (%) after incubation at room temperature (20 °C). Values are the average from at least 3 experiments. (■) lower aggregation and adhesion, and (■) higher aggregation and variable adhesion (between 13% and 18%).

<table>
<thead>
<tr>
<th>Probiotic strains</th>
<th>BATH (mean±SD)</th>
<th>Aggregation (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecium IS-16183</td>
<td>25.1±8.8</td>
<td>17.4±5.0</td>
</tr>
<tr>
<td>Enterococcus faecium IS-23427</td>
<td>46.3±12.0</td>
<td>16.7±2.3</td>
</tr>
<tr>
<td>Enterococcus faecium IS-27526</td>
<td>61.9±10.0</td>
<td>14.2±2.5</td>
</tr>
<tr>
<td>Lactobacillus plantarum IS-10506</td>
<td>76.3±16.7</td>
<td>13.3±3.0</td>
</tr>
<tr>
<td>Lactobacillus plantarum IS-20506</td>
<td>10.3±6.3</td>
<td>7.2±4.2</td>
</tr>
</tbody>
</table>
Potential Probiotic Characteristics of *Lactobacillus* and *Enterococcus* Strains Isolated from Traditional Dadih Fermented Milk against Pathogen Intestinal Colonization

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% adhesion
Effect of Oxygenated Water and Probiotic Administration on Fecal Microbiota of Rats

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Oxygenated water is water with increased concentration of physically dissolved oxygen, and can perform all the same functions as the oxygen absorbed through the lungs. Several structures of human organs participate in the absorption and transportation of the oxygen, including the villi and cells containing mitochondrion in the small intestine as well as the lymph system. The aim of this in vivo study were three folds, to validate the support of oxygenated water on viability of probiotic bacteria in the gut, to suppress the fecal coliform, and to study the effect of oxygen concentration on the profile of fecal microbiota. There were one control group and three probiotic groups of 5 rats each based on strain of probiotic supplementation, control without probiotic (a0), Lactobacillus casei commercial strain (a1), Lactobacillus sp. IS-7257 (a2) and Lactobacillus sp. IS-27560 (a3). Each group was treated with three variable treatments, without oxygenated water supplementation (b0), supplemented with oxygenated water at 50 ppm (b1), and at 80 ppm (b2). Fecal samples were collected before (c0), after 3 days (c1), 7 days (c2) supplementation, followed by 3 days after returning back to normal diet (c3), analysed by culture dependent analyses for viable fecal lactic, coliform and fecal anaerobic bacteria. Supplementation of oxygenated water at 50 ppm, significantly increase fecal lactic acid bacteria of all probiotic groups after 3 and 7 days (P<0.05); 80 ppm oxygenated water tends to lower the fecal coliform (P<0.1), while oxygenated water administration gives no effect on fecal anaerobic bacteria. As a conclusion, 50 ppm oxygenated water administration significantly increased viable fecal lactic acid bacteria in probiotic groups. On the other hand, 80 ppm oxygenated water administration tends to lower the fecal coliform bacteria. No effect of administration probiotic and/or oxygenated water on viability of fecal anaerobic bacteria.

Key words: oxygenated water, probiotic, in vivo, viable fecal microbiota, dadiih
Rats administrated with probiotic *L. plantarum* IS-10506 showed significant increased of fecal lactic acid bacteria, and significant decreased of fecal coliform.
Effect of glucose and incubation temperature on metabolically active
*Lactobacillus plantarum* from dadih in removing microcystin-LR

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*L. plantarum* IS-10506 maintained viability for 25 hours, and drop by 0.5 log cycle at 30 hours. Viable cells were measured by flow cytometry.
Effect of Probiotic *L. plantarum* IS-10506 and zinc supplementation on fecal sIgA and serum zinc of Indonesian pre-school Children
Study Design

• double-blind, randomized, placebo-controlled pre-post 90 days intervention trial on 12-24 months Indonesian children

• Four groups:
  - placebo
  - probiotic
  - Zinc
  - combination of probiotic and zinc

Probiotic *Lactobacillus plantarum* IS-10506 supplemented at $2.3 \times 10^{10}$ cfu/day; zinc supplemented at 20 mg/day as zinc sulphate, equivalent to 8 mg zinc elemental
Effect of probiotic *L. plantarum* IS-10506 and zinc supplementation on humoral immune response and zinc status of Indonesian pre-school children

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**Fig. 1.** Changes in fecal sIgA (μg/g) levels in the placebo, probiotic, zinc and combination probiotic and zinc groups after 90 days of supplementation. *p* < 0.05, **p** < 0.001.
Fig. 2. Comparison of serum zinc levels (ng/mL) before and after 90 days of supplementation in each group.
Molecular Biology, Genetics and Biotechnology

Novel probiotic Enterococcus faecium IS-27526 supplementation increased total salivary sIgA level and bodyweight of pre-school children: A pilot study

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Fig. 1. The mean elevated values of total salivary IgA levels (μg/ml) based on their nutritional status, weight-for-age Z score (WAZ).

Fig. 2. Linear regression of bodyweight in both groups. Y1 represents placebo group, and Y2 represents probiotic group.
Molecular Biology, Genetics and Biotechnology

Novel probiotic Enterococcus faecium IS-27526 supplementation increased total salivary slgA level and bodyweight of pre-school children: A pilot study

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Fig. 3. Weight gain based on nutritional status, weight-for-age Z score (WAZ).
90 days treatment, 5 groups

P0
• control biscuit with control cream

P1
• fish and soy protein isolate biscuit with control cream

P2
• control biscuit with probiotics cream

P3
• fish and soy protein isolate biscuit with probiotics cream (everyday)

P4
• fish and soy protein isolate biscuit with probiotics cream (every two days)
Changes of fecal sIgA (µg/g) based on Nutritional Status

- **P0**: P0
- **P1**: P1
- **P2**: P2
- **P3**: P3
- **P4**: P4

**Group of treatment**

The profiles of immune response in healthy subject

Orihara et. al. WAO Journal 2008, 9-14

• Lactobacillus plantarum IS-10506, and Enterococcus faecium IS-27526 are potential novel probiotics from dadih, in vitro, in vivo and pilot study in human.

• 90 days Supplementation of Enterococcus faecium IS-27526 (10^8 cfu/day) significantly increased salivary sIgA, and bodyweight gain of children younger than 5 years (p<0.05)

• 90 days Supplementation of fish and soy protein isolate biscuit with probiotics cream Enterococcus faecium IS-27526 (10^8 cfu/day) every day and every two days, and normal biscuit with cream probiotic, significantly increased fecal sIgA, of children younger than 5 years (p<0.05)
Treg cytokines have a central role in the balancing of TH1-TH2 cytokines and influence therapeutic efficacy as well as severity of allergic asthma. The special character of probiotics efficacy is shown by its capability to enhance the TH1 and Treg without reducing level of TH2 and total-IgE. Study in animal model showed probiotics efficacy in decreasing allergic reaction was achieved by involving TLR2 and TLR 4.
• The supplementation of microencapsulated *Lactobacillus plantarum* IS-10506 at $10^{10}$ cfu/day significantly increased fecal sIgA in probiotic group ($p<0.01$) and in combination of probiotic and zinc group ($p<0.027$) after 90 days to children aged 12-24 months. No adverse event was observed in both *in vivo* and in apparently healthy children, and immunocompromised children (undernourished and HIV infected ones).

• Supplementation of zinc and probiotic to children aged 12-24 months increased serum zinc concentration ($p<0.05$) after 90 days supplementation.

• Probiotic *L. plantarum* IS-10506 supplementation to children with HIV receiving ART had no significant effect on systemic immune response, but significantly increased humoral mucosal immune response.
The gut microbiota is generally important for maintaining good health.

Probiotic may improve nutrition absorption and enhancing humoral intestinal immune response.

Indigenous probiotics (\textit{Lactobacillus plantarum} IS-10506 and \textit{Enterococcus faecium} IS-27526) have been exposed to microbes surrounding, more suitable for Indonesian people.

Recommendation of $10^8$ and $10^{10}$ CFU/day as effective dose, \textit{E. faecium} IS-27526 and \textit{L. plantarum} IS-10506, respectively. No adverse effect observed.
Wonderful Team work

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which bacteria do we eat today?

Let food be your medicine and medicine be your food

Your kind attention is highly appreciated