FORMULATING PROBIOTIC PRODUCTS TO DELIVER HEALTH BENEFIT

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(Principal Nutrition Scientist)
Pharmavite LLC
OUTLINE

• Introduction to probiotics for health and wellness

• Key considerations and challenges in probiotic product formulation
  o Strain selection and identification
  o Dose Recommendations
  o Delivery form selection
  o Excipient considerations
  o Stability studies
  o Product packaging

• Developing a scientific dossier to support label claims
  o Generation of scientific support
  o Product claims

• Proper labelling of Probiotic products
OLD MODEL OF HEALTH – REACTIVE APPROACH

Physician & insurance

Relapse

Clinical Tests

Medication
NEXT GENERATION CONSUMER
A NEW MODEL FOR HEALTH – PROACTIVE APPROACH

- Diet
- Genetics
- Microbiome
- Supplements
- Exercise
- Meditation
- Self-Tracking
THE ERA OF MICROBIOME

Probiotics for Healthy People

Support a healthy microbiota – add live microbes, and the food they eat, to your diet

Consuming live microbes may help your immune system develop properly and help sustain a robust microbiota as you age. Include as part of your healthy diet:

- Fermented foods that contain live microbes
- Probiotic-containing foods
- Prebiotics and fiber, which can feed your microbes

ISAPP infographic

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PROBIOTICS MARKET

Global Probiotics Market By Region

Source: Maximize Market Research Pvt. Ltd.

Probiotics $Vol, xAOC

Millions

2YA $858.0
YA $867.7
L52W $864.0

Probiotics $Vol, xAOC

Source: Nielsen Scantrack, 52W thru 9/07/19
PROBIOTICS BEYOND GUT HEALTH

RATIONAL APPROACH TO FORMULATE PROBIOTIC PRODUCTS
WHAT IS A PROBIOTIC?

The Food and Agriculture Organization (FAO) and World Health Organization (WHO) have defined probiotics as **live microorganism** cultures which **offer health benefits** to the host when they are consumed in sufficient quantities.

NAT. REV. GASTROENTEROL. & HEPATOL., 11, 506-514 (2014)
PROBIOTIC ISOLATION

**SOURCES**

- Human microbiota
- Fermented foods
- Human Milk
- Environment

**ISOLATION AND PROPAGATION IN SPECIFIC MEDIA**

- Petri dishes
- Test tubes
- Incubator
- Sterile chamber
STRAIN IDENTIFICATION

• **Phenotypic Methods**
  — Morphological analysis
    • Microscopic – gram staining, motility
    • Macroscopic – colony appearance

• **Genotypic Methods**
  — Species Differentiation
    • Amplified ribosomal DNA restriction analysis (ARDRA)
    • 16S rDNA sequence analysis
  — Strain Differentiation
    • Denaturing gradient gel electrophoresis (DGGE)
    • Whole genome sequencing (WGS)
STRAIN GROWTH AND PHENOTYPIC PROPERTIES

— Growth Characteristics
  • Aerobic/Anaerobic
  • Optimum temperature
  • Optimum pH

— Biochemical characteristics
  • Catalase production
  • Sugar Fermentation
# PHENOTYPIC PROPERTIES [EXAMPLES]

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of isolates</td>
<td>24</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Grams character</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Shape</td>
<td>C cocci</td>
<td>C cocci</td>
<td>Rod</td>
<td>Short rods</td>
<td>C cocci</td>
</tr>
<tr>
<td>Catalase test</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Citrate utilization test</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Bile salt (0.3%) hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Growth in NaCl**

- 3.0%: + + + + +
- 5.0%: + + + + +
- 7.0%: − + + + +

**Growth at different temperatures**

- 4°C: + − + + +
- 10°C: + − + + +
- 37°C: + + + + +
- 45°C: + + + + +

**Fermentation type**

- Homo
- Hetero

**Carbohydrate fermentation**

- Glucose: + + + + +
- Lactose: + + + + +
- Sucrose: + + + + +
- Xylose: + + + + +
- Dextrose: + + + + +
- Mannose: + + + + +
- D-arabinose: − − − − −
- α-Cellulose: − − − − −
- Sorbitol: + + + + +
- D-raffinose: − + + + +

+; positive; −; negative; homo, homofermentative; hetero, heterofermentative.

Frontiers in Microbiology June 2019 | Volume 10 | Article 1382

**Lactobacilli spp.**

Optimal pH for growth

PROBIOTIC CHARACTERIZATION

Production of antimicrobial compounds

Biosafety

Evidence of benefit

Adherence to Intestinal cells

Resistant to low pH and bile acids

Antibiotic sensitivity

Probiotic Characteristics

Frontiers in Microbiology, Vol.10, Article S7


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### ACID AND BILE TOLERANT [EXAMPLES]

<table>
<thead>
<tr>
<th>Probiotic strain</th>
<th>[0, 0, 0]$^b$</th>
<th>[0.13, 0.40, 0.08, 0.40]</th>
<th>[0.26, 0.80, 0.16, 0.80]</th>
<th>[0.52, 1.60, 0.32, 1.60]</th>
<th>[1.04, 3.20, 0.64, 3.20]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bifidobacterium longum</em></td>
<td>8.84 ± 0.08 cd A$^c$</td>
<td>8.32 ± 0.23 d A</td>
<td>7.36 ± 0.06 d B</td>
<td>6.31 ± 0.23 d C</td>
<td>6.27 ± 0.37 c C</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em></td>
<td>9.15 ± 0.09 ab A</td>
<td>9.16 ± 0.04 ab A</td>
<td>8.95 ± 0.10 b A</td>
<td>8.13 ± 0.04 c B</td>
<td>8.12 ± 0.11 b B</td>
</tr>
<tr>
<td><em>L. brevis</em></td>
<td>8.50 ± 0.05 e A</td>
<td>8.61 ± 0.04 cd A</td>
<td>8.66 ± 0.21 b A</td>
<td>8.74 ± 0.10 b A</td>
<td>8.00 ± 0.13 b B</td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td>9.01 ± 0.04 bc A</td>
<td>8.83 ± 0.10 bc A</td>
<td>8.02 ± 0.09 c B</td>
<td>6.21 ± 0.13 d C</td>
<td>5.31 ± 0.59 d D</td>
</tr>
<tr>
<td><em>L. paracasei</em></td>
<td>8.96 ± 0.05 bc A</td>
<td>8.50 ± 0.24 cd A</td>
<td>6.82 ± 0.22 e B</td>
<td>6.06 ± 0.24 d C</td>
<td>5.14 ± 0.24 d D</td>
</tr>
<tr>
<td><em>L. rhamnosus</em></td>
<td>9.22 ± 0.23 ab A</td>
<td>9.11 ± 0.13 b A</td>
<td>7.54 ± 0.28 d B</td>
<td>6.33 ± 0.37 d C</td>
<td>4.97 ± 0.18 d D</td>
</tr>
<tr>
<td><em>Leuconostoc mesenteroides</em></td>
<td>8.64 ± 0.18 de A</td>
<td>7.77 ± 0.19 e B</td>
<td>5.80 ± 0.25 f C</td>
<td>5.07 ± 0.12 e D</td>
<td>3.97 ± 0.36 e E</td>
</tr>
<tr>
<td><em>Pediococcus acidilactici</em></td>
<td>9.37 ± 0.12 a A</td>
<td>9.50 ± 0.09 a A</td>
<td>9.64 ± 0.15 a A</td>
<td>9.58 ± 0.21 a A</td>
<td>9.33 ± 0.06 a A</td>
</tr>
</tbody>
</table>

---

**Table 1. Survival of Lactobacillus GG and Lactobacillus bulgaricus in Gastric Juice at Various pHs**

<table>
<thead>
<tr>
<th>pH</th>
<th>0</th>
<th>30 min</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus GG</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>8.45</td>
<td>9.12</td>
<td>8.56</td>
<td>8.59</td>
<td>8.58</td>
<td>8.44</td>
</tr>
<tr>
<td>5.0</td>
<td>8.88</td>
<td>8.93</td>
<td>8.45</td>
<td>8.47</td>
<td>8.47</td>
<td>8.58</td>
</tr>
<tr>
<td>3.0</td>
<td>8.51</td>
<td>8.62</td>
<td>8.31</td>
<td>8.30</td>
<td>8.30</td>
<td>8.30</td>
</tr>
<tr>
<td>1.0</td>
<td>&lt;4.00</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Lactobacillus bulgaricus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>8.27</td>
<td>8.32</td>
<td>7.86</td>
<td>7.92</td>
<td>8.02</td>
<td>7.69</td>
</tr>
<tr>
<td>5.0</td>
<td>8.02</td>
<td>8.05</td>
<td>7.83</td>
<td>7.96</td>
<td>7.67</td>
<td>7.47</td>
</tr>
<tr>
<td>3.0</td>
<td>7.43</td>
<td>6.24</td>
<td>5.23</td>
<td>3.17</td>
<td>2.60</td>
<td>2.30</td>
</tr>
<tr>
<td>1.0</td>
<td>&lt;4.00</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Expressed as log$_{10}$ CFU/ml.

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MUCUS ADHERENCE AND PATHOGEN INHIBITION (LGG)

Pathogen inhibition by *L. rhamnosus GG*


Digestive Diseases and Sciences, Vol 37, 121-128 (1992)

| Table 5. Persistence of *Lactobacillus GG* in Feces After Discontinuing Administration |
|---------------------------------|-----------------|-----------------|
| Viable fecal counts             |                 |
| Fecal day of GG administration  | 6.5 ± 0.3*      |
| (35/37)                         |                 |
| Days after discontinuing GG     |                 |
| 3                               | 5.4 ± 0.3       |
| (11/18)                         |                 |
| 4                               | 5.5 ± 0.2       |
| (30/37)                         |                 |
| 7                               | 4.5 ± 0.6       |
| (6/18)                          |                 |

*Mean (log₁₀) ± standard error per gram feces (number of positives/total number of subjects).*
IN VITRO AND IN VIVO ANALYSIS (SCFA PRODUCTION)


Tyagi et al., 2018, *Immunity* 49, 1116–1131
SCALE UP AND PRODUCTION OF PROBIOTICS

Microorganisms 2019, 7, 83.
PROBIOTICS DELIVERY FORMS

• Dietary Supplements
  — Capsules
  — Tablets
  — Powder
  — Gummies
  — Sachets or stick packing

• Foods and Beverages
  — Juices
  — Infant Formula
  — Chocolates, crackers, cereals, granola bars etc.
  — Fermented milk products
  — Ice cream
  — Probiotic cheese
  — Other dairy applications

• Medical Devices
  — Vaginal delivery
CELL ENUMERATION

• Colony forming units (CFUs) is the most widely recognized measure of viable microbial counts.

25 – 250 per plate

Journal of Microbiological Methods 103 (2014) 9–17
DOSE RECOMMENDATION

- Recommended dose should be equivalent or more than what is used for generating the scientific evidence in support of the claim.

### Examples

<table>
<thead>
<tr>
<th>ADULT Disorder, action</th>
<th>Probiotic strain, prebiotic, synbiotic</th>
<th>Recommended dose</th>
<th>Evidence level*</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment of acute diarrhea in adults</td>
<td><em>Lactobacillus paracasei</em> B 21060 or <em>L. rhamnosus</em> GG</td>
<td>$10^9$ CFU, twice daily</td>
<td>3</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td><em>Saccharomyces boulardii</em> CNCM I-745, strain of <em>S. cerevisiae</em></td>
<td>$5 \times 10^9$ CFU/capsule or 250 mg twice daily</td>
<td>2</td>
<td>[9,10]</td>
</tr>
<tr>
<td>Antibiotic-associated diarrhea</td>
<td>Yogurt with <em>Lactobacillus casei</em> DN114, <em>L. bulgaricus</em>, and <em>Streptococcus thermophillus</em></td>
<td>$\geq 10^{10}$ CFU daily</td>
<td>1</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td><em>Saccharomyces boulardii</em> CNCM I-745</td>
<td>$5 \times 10^9$ CFU/capsule or 250 mg twice daily</td>
<td>1</td>
<td>[11,12]</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus reuteri</em> DSM 17938</td>
<td>$1 \times 10^8$ CFU twice daily</td>
<td>3</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus acidophilus</em> NCFM, <em>L. paracasei</em> Lpc-37, <em>Bifidobacterium lactis</em> Bi-07, <em>B. lactis</em> Bi-04</td>
<td>1.70$^{10}$ CFU</td>
<td>2</td>
<td>[14]</td>
</tr>
</tbody>
</table>

World Gastroenterology Guideline, Probiotics and Prebiotics 2017
STABILITY DATA

• Temperature conditions as the recommended storage conditions on the label should be tested.

• Data should be generated under real time conditions to support stated shelf life.

• All stability testing methods, including proprietary testing methods, should be scientifically sound, repeatable, and reproducible.
• Storage and handling instructions should take into account individual formulations and packaging.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Storage Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refrigerated</td>
<td>2°C to 8°C</td>
</tr>
<tr>
<td>Cold</td>
<td>Not exceeding 8°C</td>
</tr>
<tr>
<td>Cool</td>
<td>8°C to 15°C</td>
</tr>
<tr>
<td>Controlled room temperature</td>
<td>20°C to 25°C</td>
</tr>
</tbody>
</table>
EXCIPIENT CONSIDERATIONS

- Pharmacologically Inert
- Non Hygroscopic
- Excellent Stability
- Good Solubility
- Low Calorific Value
- Pleasant or No Taste
- Cost effective
PRODUCT PACKAGING

- Factors affecting Probiotics
  - Water activity
  - Temperature
  - Compression during tableting

<table>
<thead>
<tr>
<th>Material</th>
<th>Moisture vapor transmission rates MVTR (g mil/100 in²/24hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET (Oriented or Stretch Blown poly-ethylene Terephthalate)</td>
<td>2.0</td>
</tr>
<tr>
<td>HDPE (High Density Polyethylene)</td>
<td>0.5</td>
</tr>
<tr>
<td>Glass</td>
<td>Near 0</td>
</tr>
</tbody>
</table>

Vials with Desiccant sleeve

Microorganisms, 7, 83 (2019)
HEALTH BENEFITS ARE STRAIN SPECIFIC

![Diagram showing interactions of E. coli Nissle 1917 with the intestinal microbiota and with the host.](image)

- **Host cell signaling**
  - Biofilm formation, mucin production, tight junction sealing
  - Immuno-modulation, e.g., enhanced IL-10 level, defensin synthesis
  - Anti-inflammatory effects, e.g., inhibition of IL-5, IL-6, IFN-γ, TNFα-stimulation of IL-8 synthesis
  - Inhibition of invasion of gut epithelial cells by Salmonella, EIEC, AIEC, Shigella, Yersinia, Listeria, Candida

**FEMS Microbiology Letters, Volume 363, Issue 19, October 2016**
GENERATION OF SCIENTIFIC SUPPORT - STRAIN MATTERS

*Gut Health
*Systemic Health
*Gut-Brain Axis
GENERATION OF SCIENTIFIC SUPPORT

- Randomized blinded clinical trials, Parallel or Crossover
- Open label clinical trials, Parallel or Crossover
- Systematic review of Randomized clinical trials
- Mechanistic studies in vitro and in vivo

CREDIBLE SCIENTIFIC EVIDENCE
PRODUCT CLAIMS

- Structure function claims are allowed but accompanied by “This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease”.

- Claims should be “truthful and not misleading”.

- Some of the qualified health claims are approved by FDA after systematic review of evidence.
LABELLING RECOMMENDATIONS

• Quantity should be expressed in colony forming units (CFUs).

• Labeled quantity should reflect the quantity at the end of the shelf life.

• Label should identify the genus, species and strain for each organism in the product.

• Proprietary blends are permitted by the law for dietary supplements. Individual strains within the blend should be listed in descending order of CFUs.

• If a claim pertaining to individual strains or blend of strains is made, evidence of the amounts provided in the product should be consistent with the scientific evidence in support of the claim.
THANK YOU
Questions?