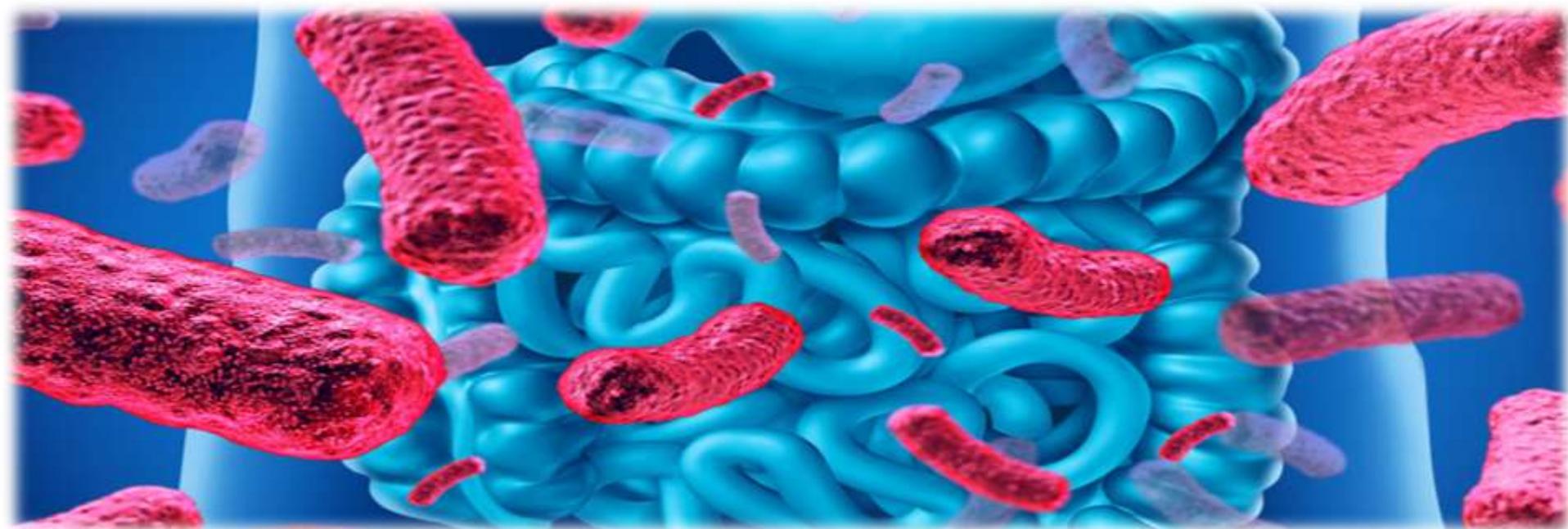


# Lactic acid bacteria as probiotics in development of health foods

# Probiotics

- Fermented milks and dairy products containing beneficial or “probiotic” cultures, such as lactobacilli and bifidobacteria, are currently among the best-known examples of functional foods.
- Defined as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host”.
- Propionibacteria, *Leuconostoc*, pediococci, and enterococci, are candidate probiotic cultures.



# What makes a bacteria probiotic???

- Survive both stomach acids (pH as low as 1.5) and bile acids (pH as low as 2).
- Bacteria must arrive in the intestines in sufficient quantities to have an effect.
- Bacteria may need to adhere to the wall of intestine (i .e. “implant”) and colonize.
- Bacteria must possess beneficial effects on human health such as **alleviation of lactose intolerance, prevention and treatment of diarrhea, maintenance of normal intestinal flora, antagonism against pathogens, stimulation of immune system, anticarcinogenic activity and reduction of serum cholesterol levels.**



BIFIDOBACTERIUM



LACTOBACILLI

## Key and desirable criteria for the selection of probiotics in commercial applications

| <b>General</b>                   | <b>Property</b>   |
|----------------------------------|---|
| Safety criteria                  | Origin<br>Pathogenicity and infectivity<br>Virulence factors—toxicity, metabolic activity and intrinsic properties, i.e., antibiotic resistance   |
| Technological criteria           | Genetically stable strains<br>Desired viability during processing and storage<br>Good sensory properties<br>Phage resistance<br>Large-scale production  |
| Functional criteria              | Tolerance to gastric acid and juices<br>Bile tolerance<br>Adhesion to mucosal surface<br>Validated and documented health effects  |
| Desirable physiological criteria | Immunomodulation<br>Antagonistic activity towards gastrointestinal pathogens, i.e., <i>Helicobacter pylori</i> , <i>Candida albicans</i><br>Cholesterol metabolism<br>Lactose metabolism<br>Antimutagenic and anticarcinogenic properties |

***Lactobacillus species***

|   |  |  |
|---|--|--|
| <i>Lb. acidophilus</i><br><i>Lb. amylovorus</i><br><i>Lb. crispatus</i> | <i>Lb. gasseri</i><br><i>Lb. johnsonni</i><br><i>Lb. casei</i> | <i>Lb. paracasei</i><br><i>Lb. rhamnosus</i><br><i>Lb. plantarum</i> |
|---|--|--|

***Bifidobacterium species***

|   |   |   |
|---|---|---|
| <i>Bf. Lactis</i><br><i>Bf. longum</i><br><i>Bf. adolescentis</i> | <i>Bf. Animalis</i><br><i>Bf. bifidum</i> | <i>Bf. infantis</i><br><i>Bf. Breve</i> |
|---|---|---|

**Other lactic acid bacteria**

|   |   |   |
|---|---|---|
| <i>Lc. Lactis</i><br><i>Ln. mesenteroides</i><br><i>P. acidilactici</i> | <i>St. diacetylactis</i><br><i>St. intermedius</i><br><i>St. thermophilus</i> | <i>En. Faecalis</i><br><i>En. Faecium</i> |
|---|---|---|

**Species of lactic acid bacteria with probiotic activity**

***Lactobacillus*** and ***Bifidobacterium*** are central to both probiotic and prebiotic (non-digestible food ingredients that stimulate the growth and/or activity of certain colonic bacteria) approaches to dietary modulation of the intestinal microflora.

Association of these bacterial groups with human health

Presence in fermented foods

Regarded as safe (GRAS) status

## **Survival and antagonism effects of LAB in the gut:**

- 1. Probiotics should be viable at the time of ingestion to confer a health benefit. Survive GI tract passage and colonize the host epithelium.**
- 2. Antagonize pathogenic bacteria by reducing luminal pH, inhibiting bacterial adherence and translocation, or producing antibacterial substances and defensins.**
- 3. *Lactobacillus* produce antimicrobial compounds (bacteriocin) to contribute to their beneficial activity. Lacticin 3147 produced by *Lactococcus lactis* inhibits *Clostridium difficile* isolates in patients with IBD.**

## **LAB and mucous layer:**

- ❑ Most mucosal surfaces are covered by a hydrated gel formed by mucins. Mucins are secreted by specialized epithelial cells, such as gastric foveolar mucous cells and intestinal goblet cells.**
- ❑ Probiotics may promote mucus secretion as one mechanism to improve barrier function and exclusion of pathogens.**

# Bioactive Compounds produced by LAB

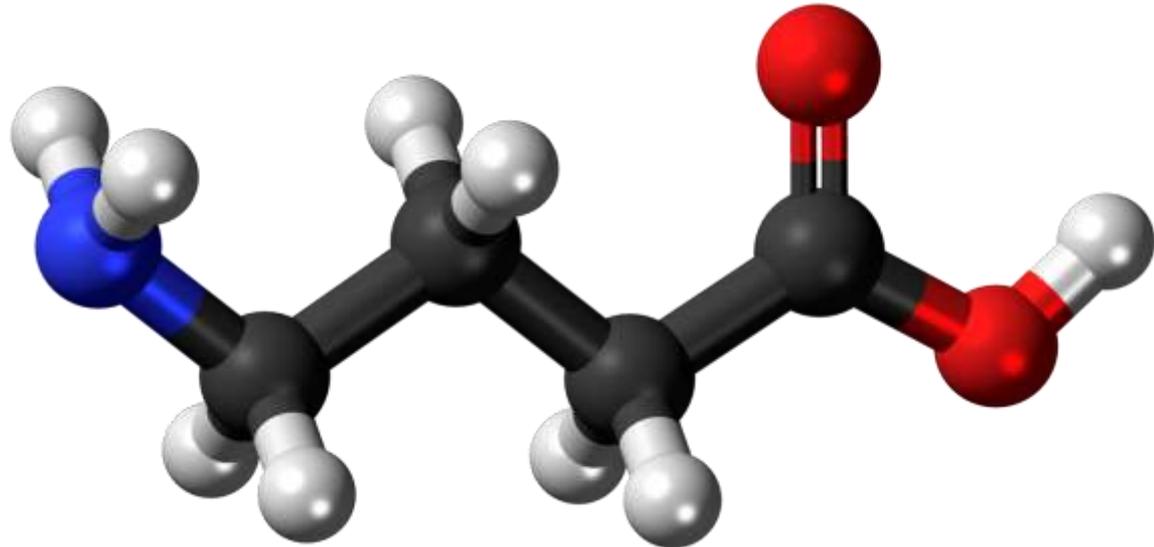
## Vitamins:

- ❑ There are 13 vitamins that must be obtained exogenously due to the inability of humans to synthesize them thus; essential nutrients in the human diet.
- ❑ *Lactobacillus* and *Bifidobacterium* can provide an additional source of B vitamins (thiamine, riboflavin, cobalamin, folate, and biotin) during dairy fermentation.
- ❑ *L. casei* KNE-1 produce thiamine and riboflavin in fermented milk drinks.
- ❑ Folate is involved in vital processes and its deficiency is generally linked to neural tube defects and poor cognitive performance. Foods can be naturally fortified with folate synthesized by LAB and bifidobacteria during manufacture of fermented foods.

| Bioactive  | Producer strain                                    | Food Product      | Health effect      |
|--|--|-------------------|--------------------|
| <b>Thiamine (B<sub>1</sub>)/Riboflavin (B<sub>2</sub>)</b> | <i>Lactobacillus casei</i> KNE-1                   | Fermented milk    | Vitamin enrichment |
|  | <i>Bifidobacterium infantis</i> CCRC14633          | Fermented soymilk | Vitamin enrichment |
|  | <i>Bifidobacterium longum</i> B6                   | Fermented soymilk | Vitamin enrichment |
|  | <i>Lactobacillus plantarum</i> CRL 2130            | Fermented soymilk | Vitamin enrichment |
| <b>Biotin (Vitamin B<sub>7</sub>)</b>                      | <i>Lactobacillus helveticus</i> MTCC5463           | Fermented milk    | Vitamin enrichment |
| <b>Cobalamin (Vitamin B<sub>12</sub>)</b>                  | <i>Propionibacterium freudenreichii</i>            | Kefir             | Vitamin enrichment |
|  | <i>Bifidobacterium animalis</i> Bb12               | Fermented milk    | Vitamin enrichment |
|  | <i>Lactobacillus reuteri</i> ZJ03                  | Soy-yogurt        | Vitamin enrichment |
| <b>Folic acid (Vitamin B<sub>9</sub>)</b>                  | <i>Streptococcus thermophilus</i><br>CRL803/CRL415 | Yogurt            | Vitamin enrichment |
|  | <i>Lactobacillus bulgaricus</i> CRL871             | Yogurt            | Vitamin enrichment |
|  | <i>Bifidobacterium lactis</i> C5CC5127             | Fermented milk    | Vitamin enrichment |
|  | <i>Bifidobacterium infantis</i> C5CC5187           | Fermented milk    | Vitamin enrichment |
|  | <i>Bifidobacterium breve</i> C5CC5181              | Fermented milk    | Vitamin enrichment |
|  | <i>Lactobacillus amylovorus</i> CRL887             | Fermented milk    | Vitamin enrichment |

## Gamma aminobutyric acid:

- ❑ GABA is the main inhibitory neurotransmitter of the central nervous system.
- ❑ Physiological functions of GABA: neurotransmission, induction of hypotension, diuretic effects etc.
- ❑ Food-grade LAB exhibit GABA-producing ability (*L. brevis*, *L. paracasei*, *L. delbrueckii*, *L. plantarum*).
- ❑ GABA has potential as a health-promoting bioactive component in foods.



## Bioactive peptides:

- ❑ During milk fermentation, LAB make use of their proteolytic system to transform milk proteins into biologically active peptides (antimicrobial, antihypertensive, immunomodulatory, and antioxidative).
- ❑ Best known ACE-inhibitory biopeptides, Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) have been identified in milk fermented by *L. helveticus*.
- ❑ Dairy starter cultures used in the manufacture of fermented dairy products (*L. helveticus*, *L. bulgaricus*, *L. plantarum*, *L. acidophilus*, *Lactococcus lactis*, or *S. thermophilus*) can generate bioactive peptides.

## Enzymes:

- ❑ Lactic acid bacteria associated to dairy fermentations possess enzymes which can be produced *in situ* during fermentation of dairy foods.
- ❑ Hydrolytic enzymes exert potential synergistic effects on digestion and alleviate symptoms of intestinal malabsorption.
- ❑  $\beta$ -galactosidase activity degrade lactose thus, improve health and reduce symptoms in lactose intolerant consumers.

## Conjugated Linoleic Acid:

- ❑ Polyunsaturated fatty acid that can be biosynthesized by LAB and bifidobacteria through bioconversion of linoleic acid.
- ❑ Health-promoting properties of CLA include anticarcinogenic, anti-inflammatory, and antidiabetic activity as well as reduce body fat.
- ❑ An effective way to increase CLA uptake in humans is to increase CLA levels in dairy products by using strains with high production potential.
- ❑ Food-grade LAB and bifidobacteria produce CLA in milk products (*L. bulgaricus* LB430 and *S. thermophilus* TA040 are suitable for production of CLA-enriched yogurt).

## Bacteriocins:

❑ Bacteriocins are ribosomally synthesized antimicrobial peptides produced by particular bacterium that are active against other competitor bacteria.

❑ These antimicrobial molecules are beneficial peptides synthesized by some LAB during milk fermentation and they have been traditionally used as naturally produced food biopreservatives.

❑ **Nisin** is the most well-known bacteriocin used as food preservative due to its antibacterial effect against *Listeria*, clostridia spores and LAB associated to spoilage.

❑ Nisin-containing Camembert and semi-hard cheeses with prolonged shelf-life were made using *Lactococcus lactis*.

# Health Effects of Probiotics

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## *Well-substantiated effects*

- Alleviation of lactose maldigestion
- Prevention/treatment of infections
- Reduction of serum cholesterol
- Chemopreventative effects
- Modulation of the immune system

## *Potential effects*

- Treatment/prevention of inflammatory bowel disease
  - Alleviation of constipation
  - Improvement of dermatitis
  - Liver disease therapy
-

| Bacteria  | Probiotic effects  | Properties  | Use  |
|---|--|---|--|
| <i>Lactobacillus acidophilus</i><br>(6 major species) | <ul style="list-style-type: none"> <li>may fight intestinal infection</li> <li>reduces intestinal transit time</li> <li>may prevent colon cancer</li> <li>may be hypocholesterolemic</li> </ul>  | <p>Survives GI transit well. (Ability to survive varies between strains.)</p> <p>Adherence demonstrated in vitro but not yet demonstrated in vivo.</p> <p>Grows slowly in fermented products; doesn't survive well in fermented products.</p> | Used in acidophilus milk and in kefir; may be used in yogurt.  |
| <i>Lactobacillus GG</i>                               | <ul style="list-style-type: none"> <li>prevents growth of pathogens</li> <li>delays tumor development</li> <li>prevents traveller's diarrhea,</li> <li>antibiotic-associated diarrhea and infant diarrhea</li> <li>alters activity of intestinal microflora</li> </ul> | <p>Implants and colonizes the intestinal tract. However, the colonization isn't permanent.</p> <p>Research shows that probiotic bacteria should be consumed a few times a week to maintain their effect on intestinal flora.</p>              | <p>Some new fermented dairy products using <i>LGG</i> are available in Europe. Minimum levels necessary for colonization are:</p> <ul style="list-style-type: none"> <li><math>10^8</math> cells in milk,</li> <li><math>10^9</math> cells in fermented milk or enteric tablets,</li> <li><math>10^{10}</math> cells in gelatin capsules.</li> </ul> |

| Bacteria                              | Probiotic effects   | Properties  | Use  |
|---------------------------------------|---|---|--|
| <i>Lactobacillus casei</i>            | <ul style="list-style-type: none"> <li>■ can increase levels of immunoglobulins, <math>\gamma</math>-interferon, and phagocytic activity</li> <li>■ decreases activity of enzymes related to the risk of colon cancer</li> <li>■ alters composition of intestinal flora, possibly by restricting growth of pathogens</li> </ul> | <p>Some strains survive intestinal transit.</p> <p>Does not colonize.</p>   | <p>Used in kefir and many cheeses, including parmesan and cheddar; also used in some new yogurt-like products.</p> |
| <i>Bifidobacteria</i><br>(29 strains) | <ul style="list-style-type: none"> <li>■ reduces incidence and duration of diarrhea</li> <li>■ reduces intestinal transit time</li> <li>■ may reduce colon cancer</li> <li>■ increases production of secretory immunoglobulins</li> </ul>   | <p>Some strains of Bifidobacteria survive intestinal transit well, but it is not clear whether they implant.</p> <p>Produce both lactic acid and acetic acid.</p> | <p>May be used in yogurt.</p>  |