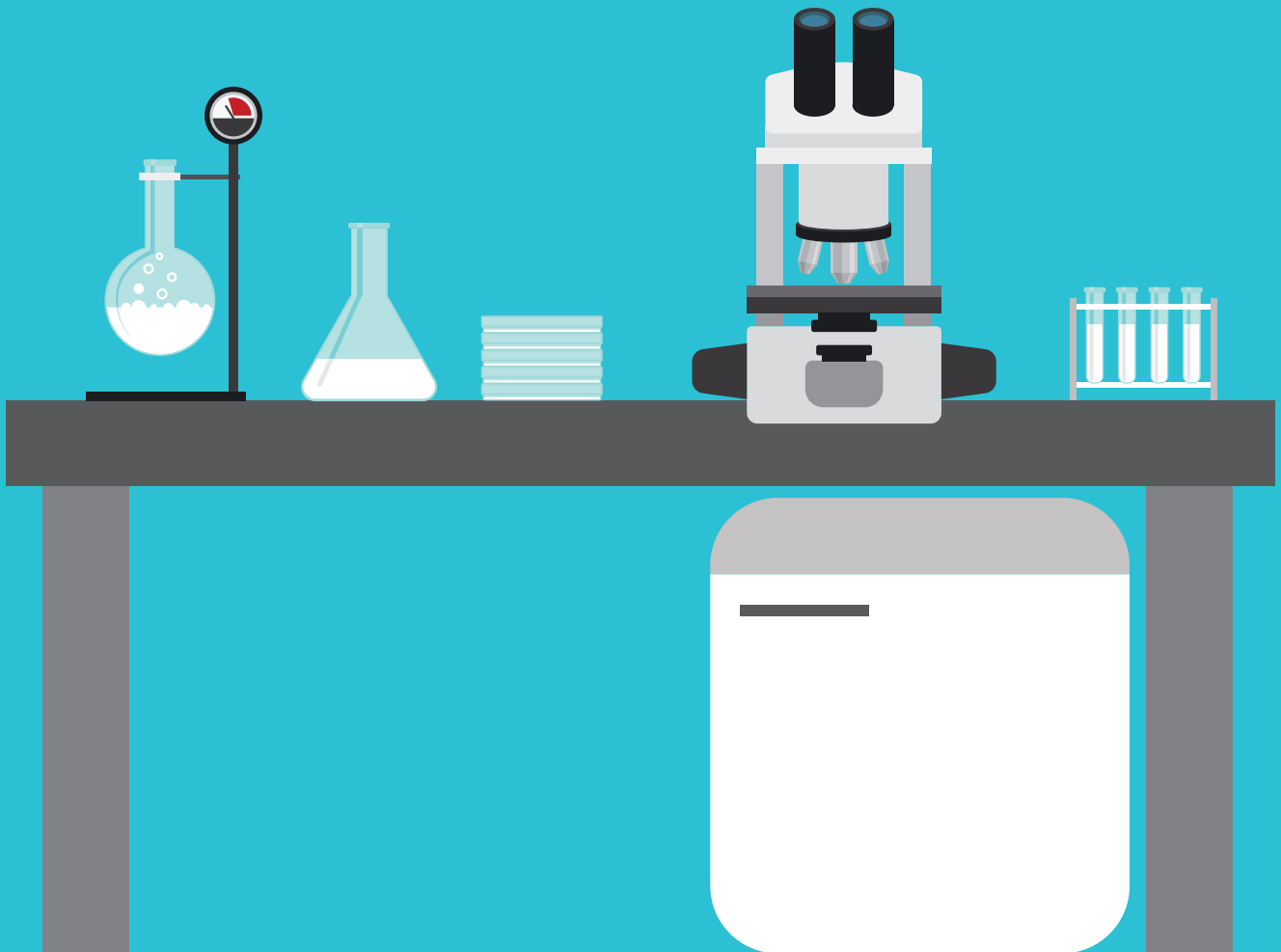


Under the Microscope



What is Yakult?

Yakult is a fermented milk drink containing our unique probiotic bacteria, the *Lactobacillus casei* Shirota strain.

Yakult

www.yakult.com.au

Bacteria We Want

Bacteria play important roles in many aspects of our daily lives. Some are useful and help produce medicines, foods and drinks while others can cause illness or decay.

Useful bacteria include:

- *Lactobacillus bulgaricus* – bacteria that turns milk into yoghurt. (lack-toe-bah-sill-us bul-ger-ri-us)
- *Streptomyces* – bacteria from soil used to make streptomycin, an antibiotic used to treat infections. (strep-toe-my-seas)
- *Pseudomonas putida* – one of the many microorganisms used at water treatment plants to clean wastes from sewage water. (sue-doe-moan-us-poo-tea-dah)

Fermented Foods:

- Used for thousands of years as a safe way to store perishable food
- Part of traditional diets in many countries, with evidence that fermented products such as beer, cider, vinegar and soy sauces date back to 4000 BC

Fermentation:

- Chemically converts carbohydrates into alcohol and acids via microorganisms such as bacteria, yeasts and moulds
- Is used to create lactic acid in sour foods such as pickles, sauerkraut, yoghurts, sour cream and fermented milk drinks
- Helps preserve meats such as salami and prosciutto

Fermented foods are popular not just because of their taste and flavour, but also for prolonging shelf-life, nutritional value and health promoting properties. Fermentation has five main roles in food production:

1. Developing flavour, texture and aroma
2. Preserving
3. Improving nutrient availability
4. Inhibiting undesirable microorganisms
5. Reducing cooking time

The fermentation process changes the physical and chemical properties of the original food and has been shown to improve the digestibility of carbohydrates and proteins, as well as increasing the levels of some vitamins and the availability of certain minerals.



Examples of fermented food

Lactic Acid Bacteria

- Lactic Acid Bacteria (LAB) were discovered by Louis Pasteur in 1857 and are recognised as beneficial bacteria closely associated with digestive balance.
- Elie Mechnikov discovered 'healthy bacteria' in 1908 and developed a theory that ageing is caused by toxic bacteria in the gut and concluded that lactic acid could prolong life. Based on his theory, he drank sour milk every day.
- The term Lactic Acid Bacteria refers to bacteria that utilise sugars, such as glucose or lactose, to produce lactic acid via fermentation.

Microbes for Intestinal Health

Bacteria play a vital role in the digestive system; without them, our systems would not function correctly. The key to optimal digestive function is maintaining 'digestive balance' so numbers of beneficial bacteria outnumber those that are potentially harmful.

Digestive balance can be disrupted by a number of lifestyle factors which deplete the number of beneficial bacteria in the digestive system. These include stress, an unbalanced diet, the natural ageing process and some medications, particularly antibiotics. Digestive imbalance is associated with a range of undesirable digestive symptoms and some digestive disorders.

Beneficial Bacteria



Lactobacillus



Bifidobacteria



Lactococcus

Potentially Harmful Bacteria



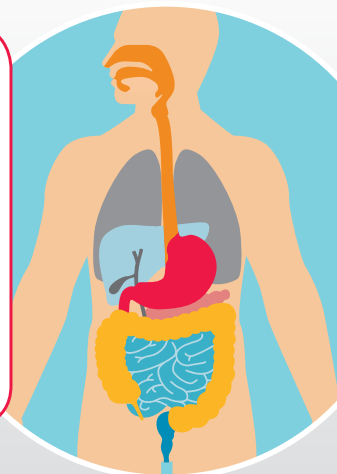
E.coli



Staphylococcus



Clostridium Perfringens



Beneficial bacteria play a number of important roles in the digestive system including:

- Helping to control the numbers of potentially harmful microorganisms and the toxins they produce
- Regulating bowel movements
- Assisting with digestion and absorption of food
- Synthesising vitamins
- Producing short chain fatty acids
- Stimulating the immune system

What are Bacteria?

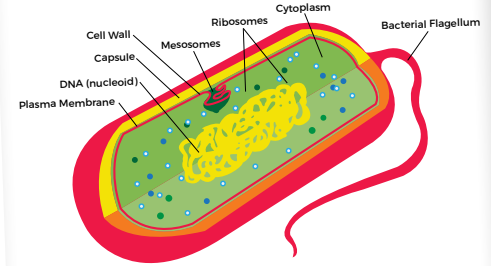
Bacteria are microscopic living organisms, single-celled and can be found everywhere on Earth. There are harmful bacteria which can be pathogenic causing disease in some living organisms and beneficial bacteria which can help improve or maintain health in some living organisms, such as the bacteria used in Yakult.

Bacteria can also play an important role in processes such as fermentation and decomposition.

Bacteria are:

- The earliest form of life found on Earth that can reproduce
- Classified according to important features such as size, shape, structure, arrangement, presence of flagella, photosynthetic material and growth patterns
- Only observable under a microscope
- Prokaryotic cells (single celled organisms with no nucleus) with cytoplasm containing chromosomal material surrounded by a cell membrane and wall
- Sensitive to:
 - Physical effects of temperature or pressure
 - Presence of oxygen and light
 - Availability of nutrients
 - Exposure to toxins
 - Biological influences of coexisting species
- Able to use energy sources from sugars, starches, sunlight, sulphur and iron
- Able to survive in a diverse range of environmental conditions; from freezing conditions inside Antarctic ice to extreme heat within volcanic lava
- Generally named by their genus, species and strain name

All bacteria need their own combination of nutrients and environmental conditions for survival and optimal growth otherwise they remain dormant or die.



General Structure of a Bacteria

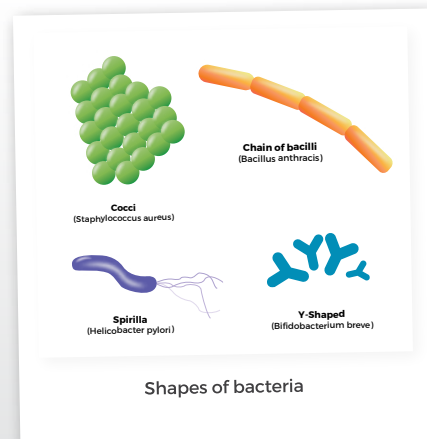
Size

Bacteria are microscopic, varying in length between 0.1 -10µm, and require a highly powered microscope to be seen. The below images demonstrate that even at 30 times magnification, bacteria are unable to be identified on the tip of the needle. Using an electron micrograph at 3000 times normal vision allows for individual bacteria to be viewed.

Shape

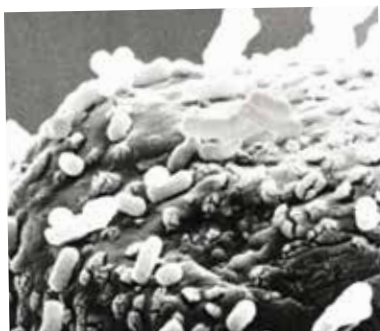
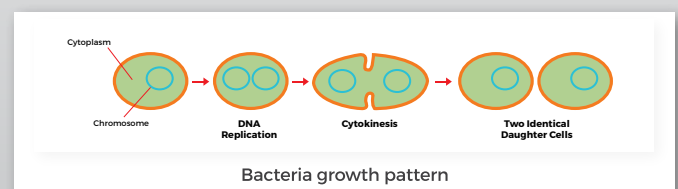
Grouped according to shape, bacteria can be;

- 'bacilli' or rod-shaped - eg *Bacillus anthracis*.
- 'cocci' or spherical-shaped - eg *Staphylococcus aureus*.
- 'spirilla' or spiral-shaped - eg *Helicobacter pylori*.
- V- or Y-shaped - eg *Bifidobacterium breve*.



Growth patterns

A single bacterium reproduces by a process called binary fission. This process divides a single cell into two cells with the replication of contents within the cell. The time taken to replicate one single cell can be used to assist in classifying bacteria.



Individual bacteria are more distinct, magnified x3000
image sourced from Yakult Honsha



Unknown bacteria on tip of needle, magnified x30
image sourced from Yakult Honsha



What is in Yakult?

Every 65ml bottle of Yakult contains 6.5 billion live *Lactobacillus casei* Shirota strain. The strain is cultured under precise conditions and rigorously tested to ensure high numbers of 'Colony Forming Units' (or the number of bacteria present) are present through all stages of manufacturing.

What are Probiotic Bacteria?

Probiotic bacteria are defined as live microorganisms that, when taken in adequate amounts, provides a health benefit to the host. The benefit of a probiotic is specific to that strain of bacteria. Strains belonging to the *Lactobacilli* and *Bifidobacteria* species are the most widely researched probiotic species. These bacteria are used in foods such as fermented milk drinks and yoghurts. Probiotic bacteria are available from a number of different sources including fermented milk drinks, yoghurts, capsules and powders.

To be considered 'probiotic' these bacteria must meet strict scientific criteria such as being:

- Safe for human consumption
- Able to survive the:
 - Production process
 - Product shelf life
 - Journey through the strong digestive juices to reach the intestines alive
- Alive and viable in the final product
- Present in the final product at a high concentration
- Able to confer a health benefit which has been substantiated by research

What's in a name?

- Lacto- the bacteria's ability to ferment sugars to produce lactic acid
- bacillus- distinctive rod shape
- casei- from casein, a protein found in dairy products
- Shirota- name of the strain discovered by Yakult's founder, Dr Minoru Shirota, and named in his honour

Lactobacillus casei Shirota strain:

- Is rod shaped and approximately 0.5 μm in diameter and 2.0 μm in length
- Produces lactic acid as the predominant product of fermentation when grown in a suitable medium with glucose and lactose as the major carbohydrate source
- Is highly acid resistant and able to survive the journey through the gastric juices and bile to arrive alive in the small intestine, where it helps to maintain the balance between beneficial and potentially harmful bacteria
- Encourages the growth of beneficial bacteria already present in the intestines
- Suppresses the growth of harmful bacteria and the toxins they produce



Lactobacillus casei
Shirota strain

Yakult's Quality Control (QC)

- QC activities involve sampling, testing and inspection of the product, bottles and packaging. This confirms the Quality Assurance (QA) measures have been effective.
- Individual bottles are randomly inspected along the production line to check for incorrect printing, undesirable markings and lid sealing.
- Approximately 100-200 product samples are collected and tested for every batch of Yakult created.
- Raw ingredient samples are tested for quality prior to purchasing a batch.
- The high quality of Yakult is ensured through an extensive variety of tests that include microbiological quality, composition and taste. Once approved, the product is ready to be released to stores.



QC testing includes more than 200 assessments comprising:

1. Specific Gravity – measures the density of a liquid using a densometer.
2. Brix – measures the sugar concentration of foods using a refractometer.
3. Titratable Acidity – measures the level of acid development in the product and is used to monitor growth numbers of *Lactobacillus casei* Shirota strain.



Microbiological tests which ensure there is no microbiological contamination include:

4. Lactobacillus Enumeration – measures the number of Shirota strain in a sample at the end of the fermentation process. The Shirota strain is plated on a selective agar and cultured using specific inoculation times and temperatures. In combination with a dilution method, Lactobacillus enumeration indicates the number of Shirota strain in the final product expressed as Colony Forming Units (CFU).
5. Standard Plate Count (SPC) – measures the number of non-Lactobacillus bacteria in a sample, measured by growth on selective agar at incubation temperatures and expressed as CFU. SPC is used to indicate the presence and level of contaminating bacteria.
6. Yeast and Mould numbers are determined through plated samples and expressed as Yeast and/or Mould CFU.
7. Coliform numbers are determined by the growth of a sample on a selective agar at incubation temperatures and are expressed as Coliform CFU. Coliform numbers indicate contamination, resulting from inadequate food handling.

Extensive sampling and testing throughout all stages of manufacture enable Yakult Australia to ensure:

- Production of consistently high quality products.
- Presence of a single strain of probiotic bacteria in each bottle – 6.5 billion *Lactobacillus casei* Shirota strain.
- Absence of contaminating bacteria, yeasts, moulds and coliforms.



Food Safety

The Australian New Zealand Food Standards Code incorporates legislation associated with the production, labelling and marketing of foods in Australia. The Code also covers food safety information and current procedures for food handling- from the handling of raw ingredients right through to consumption.

Yakult's manufacture requires documented precautions and procedures to ensure the product is not subject to any hazards that might compromise its safety, hence the use of Hazard Analysis and Critical Control Points (HACCP).



HACCP

- Identifies potential risks at every point of manufacture
- Prevents and controls potential risks
- Implements corrective action
- Monitors all processes

At Yakult our commitment to quality extends to transportation. We ensure the cold chain is uninterrupted and the temperature in Yakult's refrigerated vans and transport delivery vehicles is between 0-4°C.

There are 7 steps in a HACCP Plan.

1. Hazard Analysis and Risk Assessment

Develop flowchart for the manufacturing process.

2. Identification of critical control points

Identify potential hazards; these could be physical, chemical or biological.

3. Control standards for critical control points

Set measurable standards for each critical control point.

4. Monitoring of critical control points

Regular or scheduled monitoring of each critical control point.

5. Corrective action

Actions to be taken in the event of critical control point being reached.

6. Record keeping system for HACCP program

Keep all monitoring records of critical control points and critical limits. Corrective action is documented if taken.

7. Review of HACCP program

Documentation gets audited by external auditors every year. Random checks can also occur.

Yakult

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