The ingredients of beer and Kirin’s commitment

We use water that has been refined with Kirin's proprietary water treatment technology and has passed rigorous screening standards

We use yeast selected specifically for each product from a bank of about 1,000 yeast varieties

Made from selected barley meeting numerous quality standards

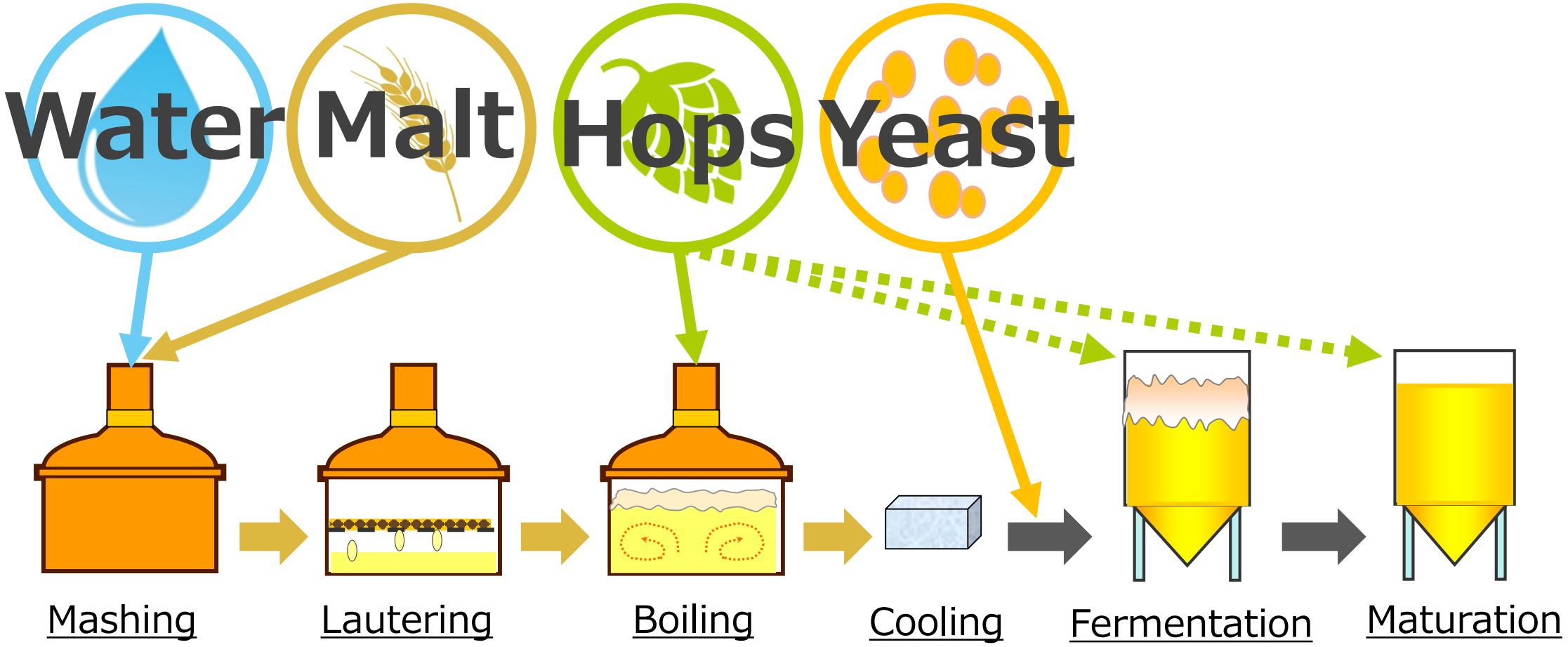
➡ **The only beer brewery in Japan with in-house malting (Fukuoka)**

Every year, our experts visit hop-producing areas in Japan and abroad, and use all five senses to select the best hops

Japanese hops

➡ **We use about 70% of hops produced in Japan (Tohoku region)**

The brewing process



Malt provides nutrients for yeast fermentation while at the same time giving the beer its flavor and aroma

Malt = sprouted barley

Sprouting generates the enzymes necessary for growth

➡ These enzymes are important in brewing

Types of grains

- Barley is most commonly used
- Wheat and other ingredients are also used in some beers, including craft beers

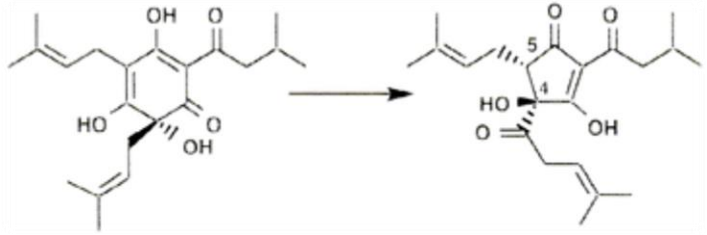
Degree of roasting malt

- Dark beers have a higher percentage of roasted dark malt
- The use of dark malt gives dark beers a savory, smoky flavor

The role of hops in brewing beer

Bitterness

- The alpha acids in the hops added during the wort boiling process are isomerized to become iso-alpha acids (the main source of bitterness).

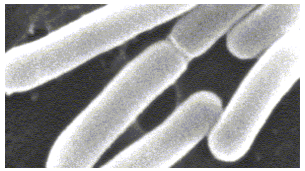


Aroma

- A range of aromas may be produced depending on the variety of hops and the timing of their addition

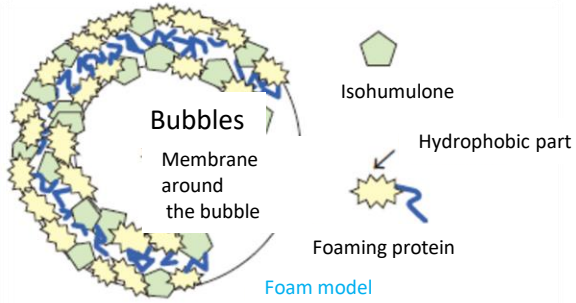
Antibacterial action

- Inhibits the growth of haze-producing bacteria, such as lactic-acid bacilli, and improves the shelf life of beer



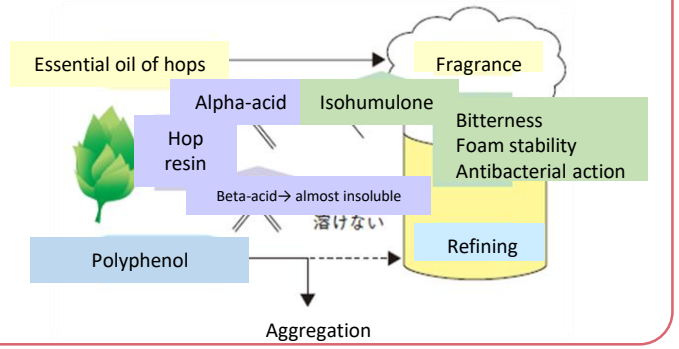
Foam formation

- Iso-alpha acids act as an important factor in foam formation, and contribute to foam quality



Stability

- Solidifies and refines excess protein in the wort



Kirin has developed a range of proprietary technologies to control beer ingredients

Creating New Raw Materials

- Creating a diverse range of flavors through selective breeding of raw materials

Creating a New Production Method

- Driving the evolution of flavor with innovations in raw material processing and manufacturing processes

Creating New Value

- Utilizing ingredients derived from raw materials to add health-promoting properties

Technology employing frozen raw hops (produced in Japan)

Usually dried after harvest

Pellets

- Excellent storage stability, transportability and handling at production sites

Freezing

Shipped “raw” on the day of harvest

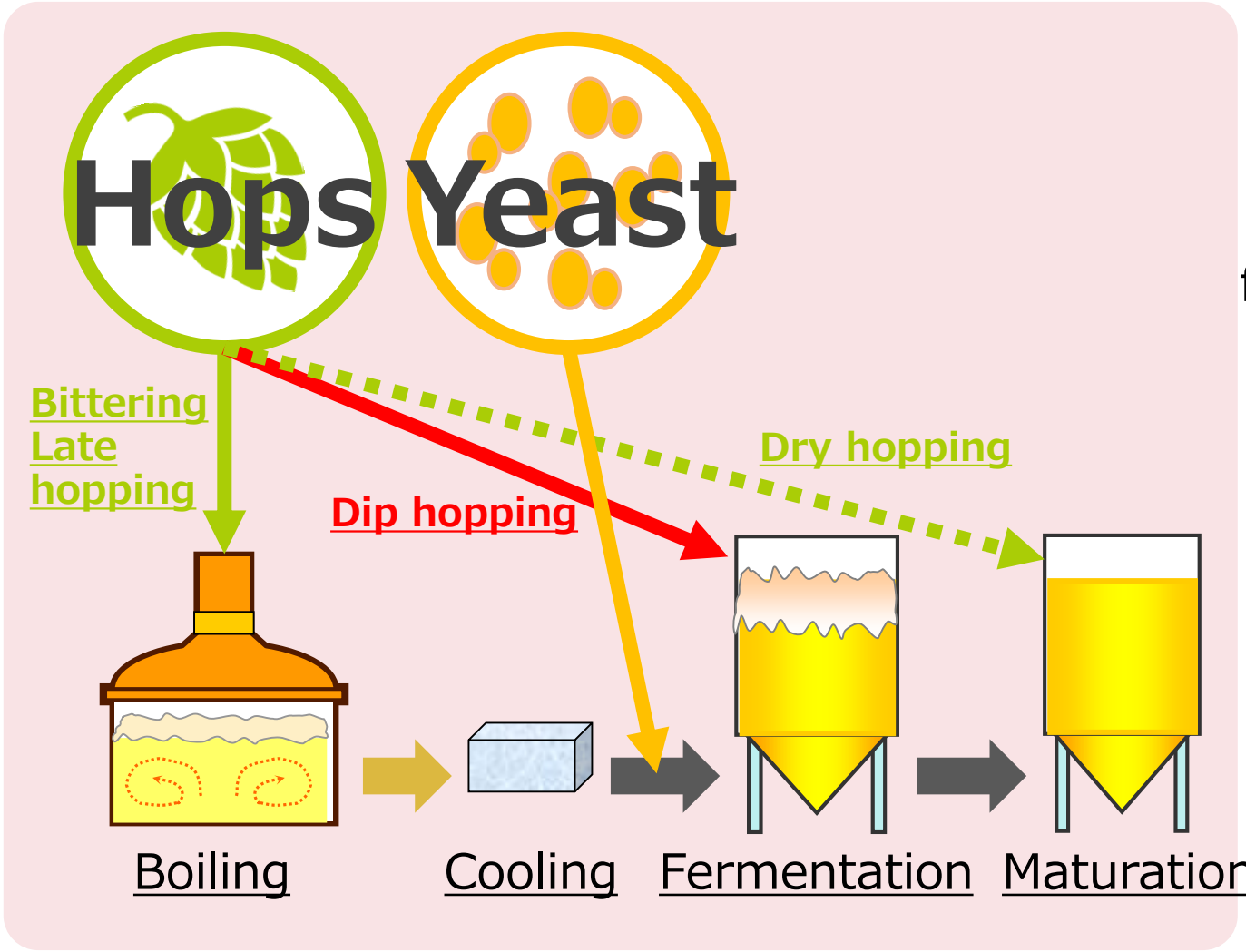
Freezing

Frozen milling

“Raw” hops are placed into the machinery manually

17th year on the market!

Kirin’s Original Dip hopping

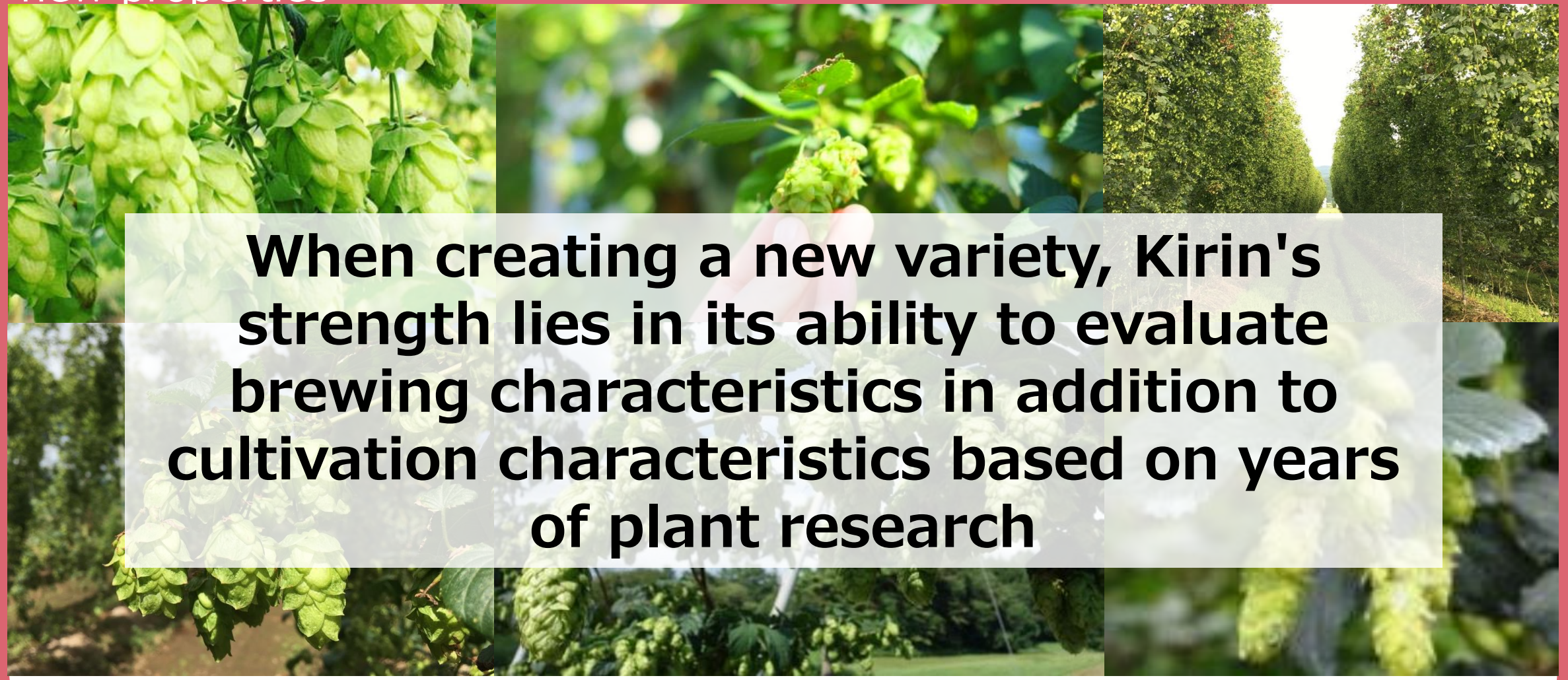


Dip hopping technology

Dipping the hops during fermentation suppresses excessive bitterness and pungent odors while leaving plenty of pleasant hop aroma

What does breeding/variety improvement consist of?

Breeding is the creation of genetic populations (= new varieties) with new properties



When creating a new variety, Kirin's strength lies in its ability to evaluate brewing characteristics in addition to cultivation characteristics based on years of plant research

Kirin's original variety, MURAKAMI SEVEN

Kirin is uniquely capable of creating shared value (CSV) by simultaneously adding value to its products (thus improving profitability) and maintaining the production of hops in Japan (thus revitalizing local economies)

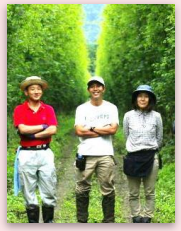
MURAKAMI SEVEN

Appeal for brewers (brewing characteristics)

A unique aroma unlike any other in the world

➡ “figs, muscats, Japanese citrus fruits”

Appeal for producers (cultivation characteristics)



Excellent cultivation properties

➡ “Allows high yields and labor-efficient cultivation”

Background of the technology

We started researching various plants basing on the research experience of barley and hop.

Breeding and improvement of hop and barley varieties

×

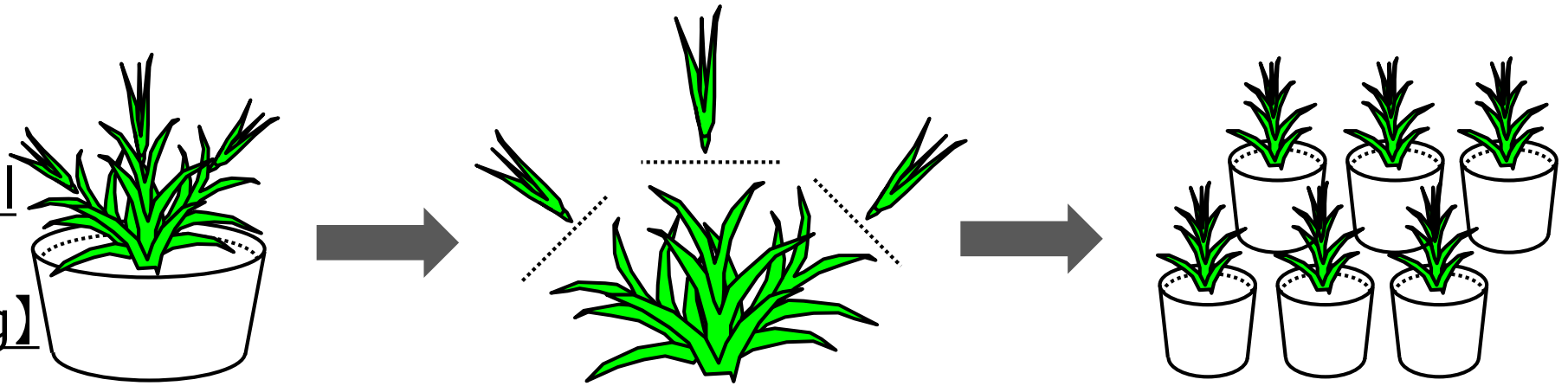
Biotechnology

Development of technology for large-scale plant propagation

Overview of the technology

High-quality, uniform plant production requires the growth of plants with the same characteristics as the parent plant. This is usually achieved via “plant cutting”.

Conventional method:
【plant cutting】



Parent plant

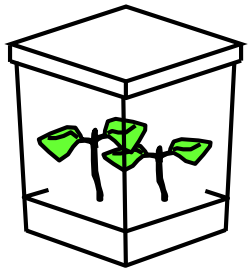
Placed one by one by hand‥

Same plant as parent plant

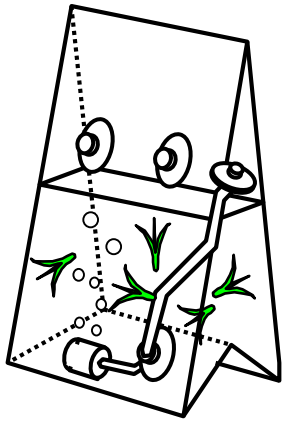
Characteristics of this technology (1)

“Our bag-based cultivation technology” is overwhelmingly more efficient than the use of “plant cuttings”

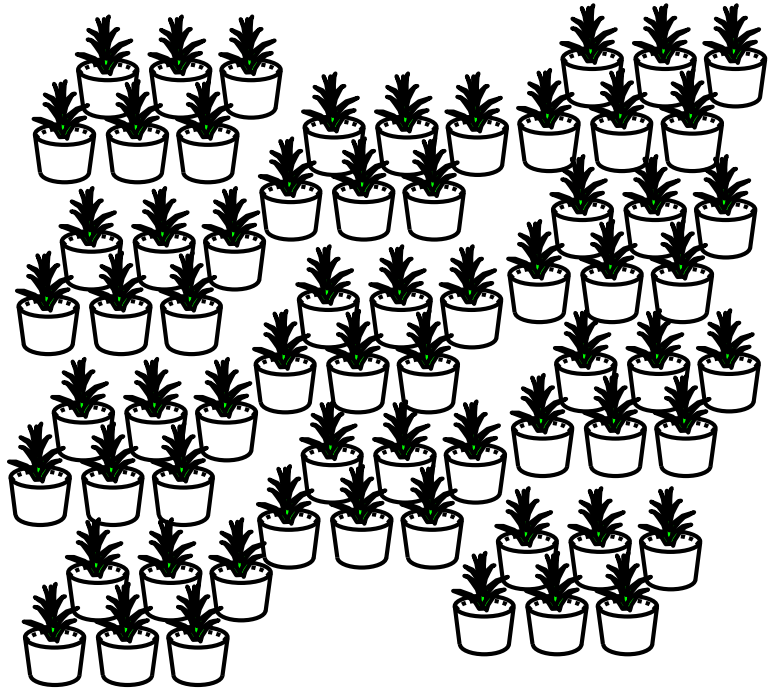
Kirin's unique bag-based cultivation technology



Parent plant



Culture in liquid inside unique bags



Same plant as parent plant

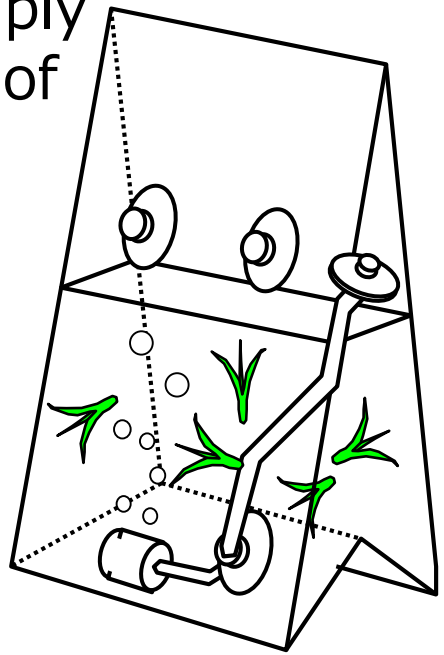
Characteristics of this technology (2)

Kirin's unique technology for growing plants inside bags has various distinguishing features

Production volume can be freely adjusted by simply changing the number of bags

Allows preserving sterility

Cheaper than tanks



Easy to work with

Small and easy to handle

Light and safe

Uses of this technology (1)

Mass production of carnations established the new business model for Mother's Day in Japan

Bag-based cultivation

- Small enough to hold in one hand
- Grow inside sterile bags
- Makes it easy to increase and adjust production by changing the number of bags

Transplantation in greenhouse

- Uniform, high-quality growth
- High survival rate

Just before shipping

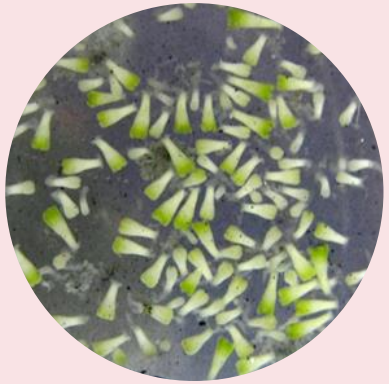
- The flowering period is synchronized, allowing shipping by pallet

Uses of this technology (2)

High efficiency helped recover seaside protection forests affected by the earthquake

A seaside protection forest that was severely damaged by the Great East Japan Earthquake

Collection of pine cones



Disease-resistant black pine grown with bag-based cultivation technology

Seedlings

Seedlings for forestation

(Partner organizations: Forest Research and Management Organization, others)

Uses of this technology (3)

Also used in Japanese potato production/industry

New species of pests arrive from abroad
The rapid spread of resistant varieties is urgently needed

Providing technology to the Center for Seeds and Seedlings, NARO

Disease-resistant varieties grown in sterile culture

Several hundred potatoes can be harvested from just one container numerous times each year

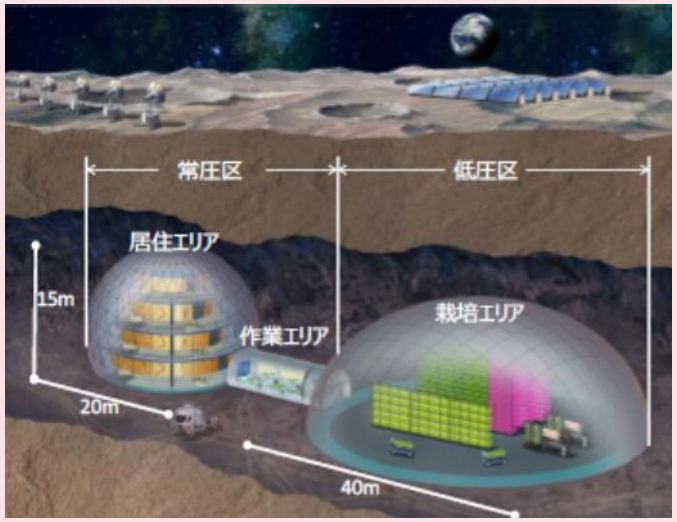
Very high productivity (red-skin variety)

Future outlook

Use in various fields conducive to the solution of social issues is expected

- Growth of plant seedlings relating to resources and energy
- Seedlings of plants in tropical and subtropical plantations (greater seedling production efficiency, rapid spread of new varieties)
- Fields where innovative cultivation methods are needed(evolution from the plant cutting-based approach)

【Case study: considering use in a space farm】



- Expected to achieve plant growth in a unique environment (space) thanks to virus-free growth, high efficiency and small-lot production, among other features

Overview of this technology

Applying the technology for bag-based plant cultivation to cells would allow the mass production of useful substances

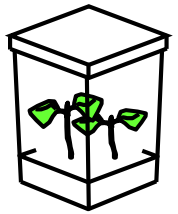
What are useful substances?

Pharmaceutical raw materials and functional ingredients

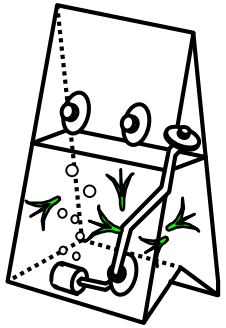
Overview of this technology

Cultivation of plants and cell proliferation

plants



An individual plant



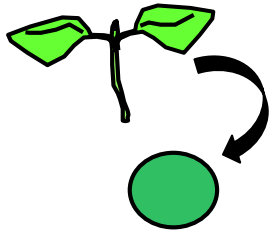
Bag-based cultivation

Propagated plants

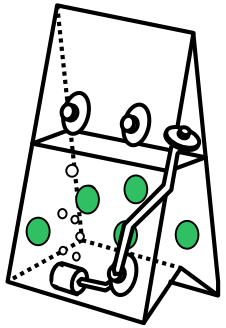
Cultivation

Agricultural products

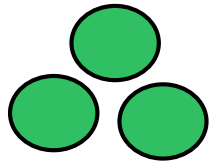
Cell



A plant cell isolated from plant



Bag-based cultivation



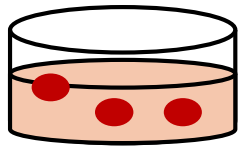
Plant cells

Refinement

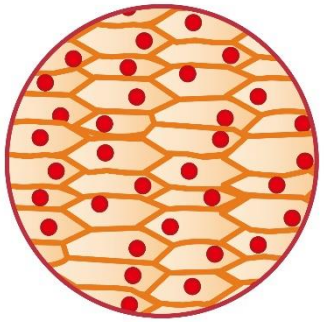
Useful substances
・Functional ingredients
・Pharmaceutical raw materials

Background of this technology

Regenerative medicine is a type of medical treatment whereby dysfunctional organs and tissues are regenerated by inserting “cells” or “genes” themselves into the body

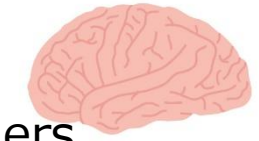


Manufacturing

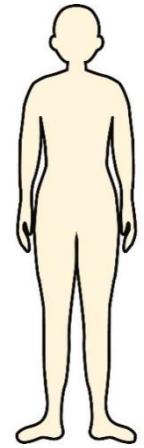


Administration

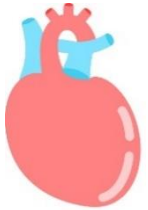
Stroke



Corneal disorders

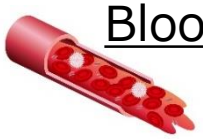


Cardiomyopathy



Severe burns

Blood cancers



etc…

iPS cells and other cells used in regenerative medicine

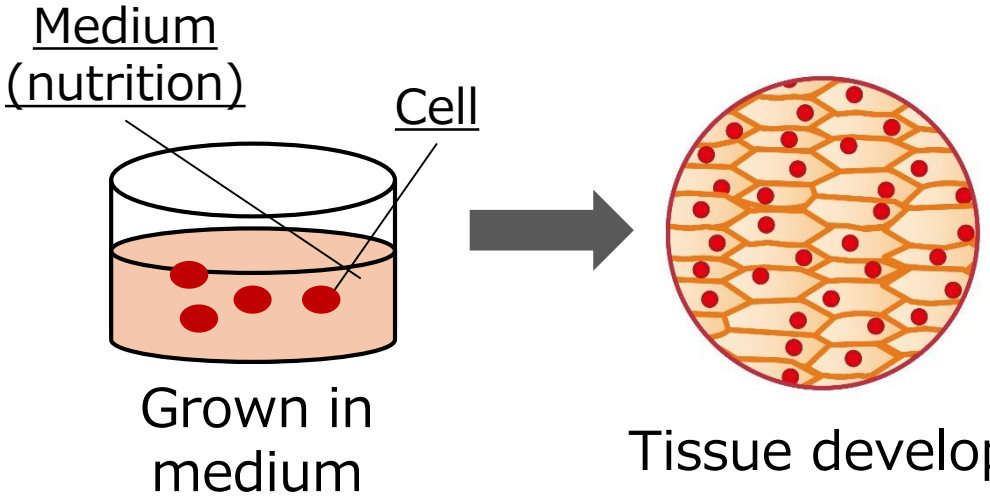
Tissue made from cells used in regenerative medicine (regenerative medicine products, etc.)

Dysfunctional organs/tissues are regenerated

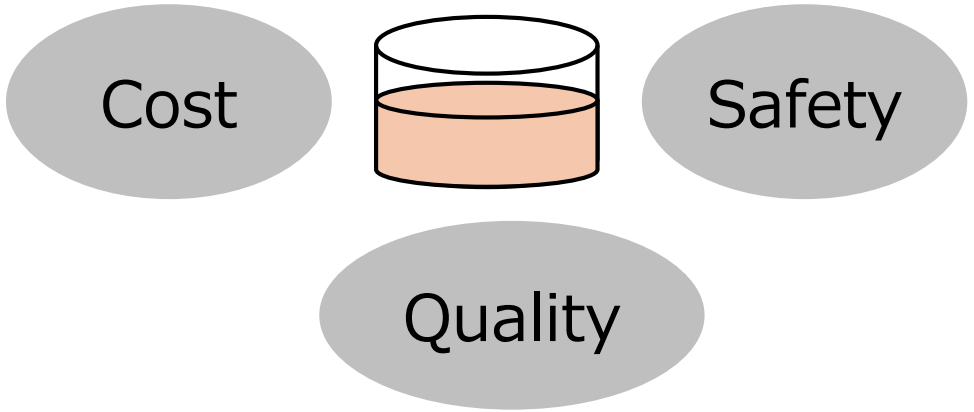
Background of this technology

Medium are essential for regenerative medicine ～Problems with medium are one of obstacles to the development of regenerative medicine

Medium to grow cells are essential



Various problems related to the medium

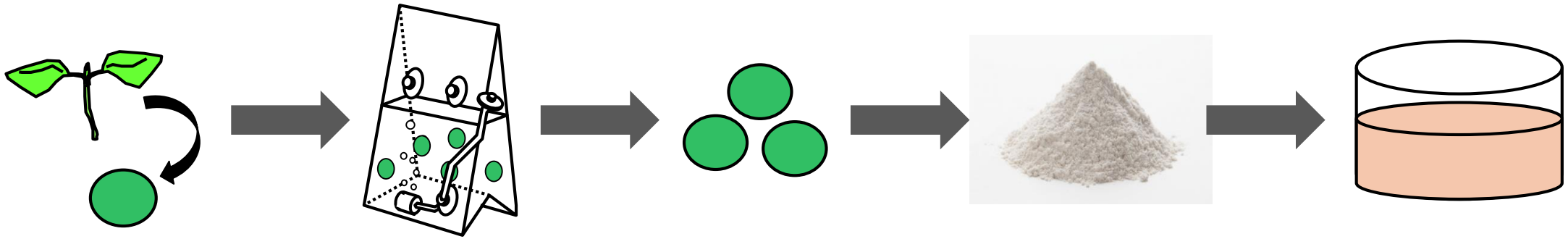


Cannot grow cells

No proper culture medium available

What can be achieved with this technology

Use of our technology for bag-based plant cell cultivation would allow producing medium for regenerative medicine meeting these challenges



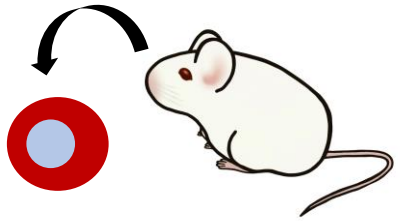
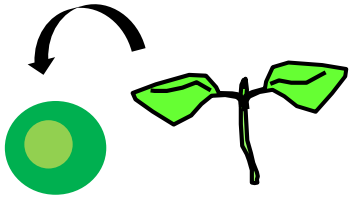
Cells taken from plant → Bag-based cultivation → Plant cells → Components of medium → Medium

All met

- Cost
- Safety
- Quality

Uniqueness of this technology

While recombinant proteins such as medium components are often made in animal cells, those made in plant cells allow for virus-free, safe, easy, and inexpensive production.

	Cost	Safety	Quality
 Animal cell	Requires expensive raw materials	Virus infection risk	Special facilities are to ensure pharmaceutical-grade quality for both animal and plant cell-derived proteins
	Inexpensive	**Safety**	
 Plant cell	**Can be produced with inexpensive raw materials**	**No viruses capable of infecting humans from plants**	**Kirin has already built these facilities** 

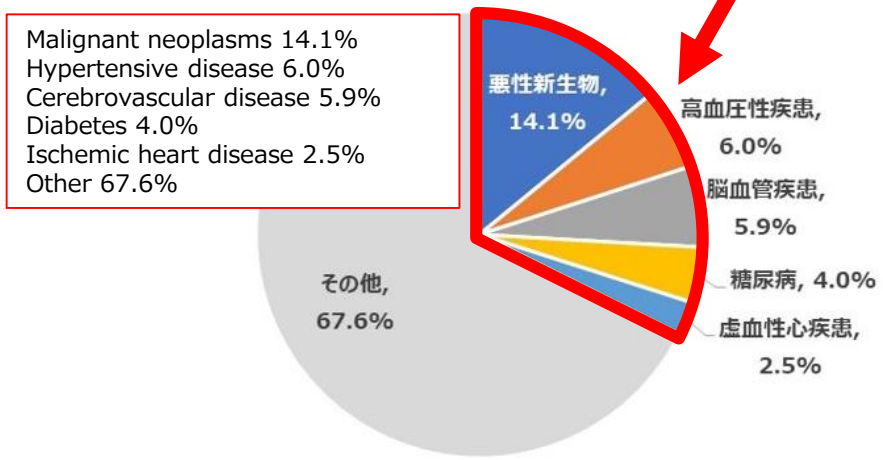
Excessive fat accumulation increases the risk of lifestyle diseases

* "Patient Survey 2014" Health Statistics Office, Policy Planning and Evaluation Division, Ministry of Health, Labour and Welfare

■17.8 million* people suffer from lifestyle diseases

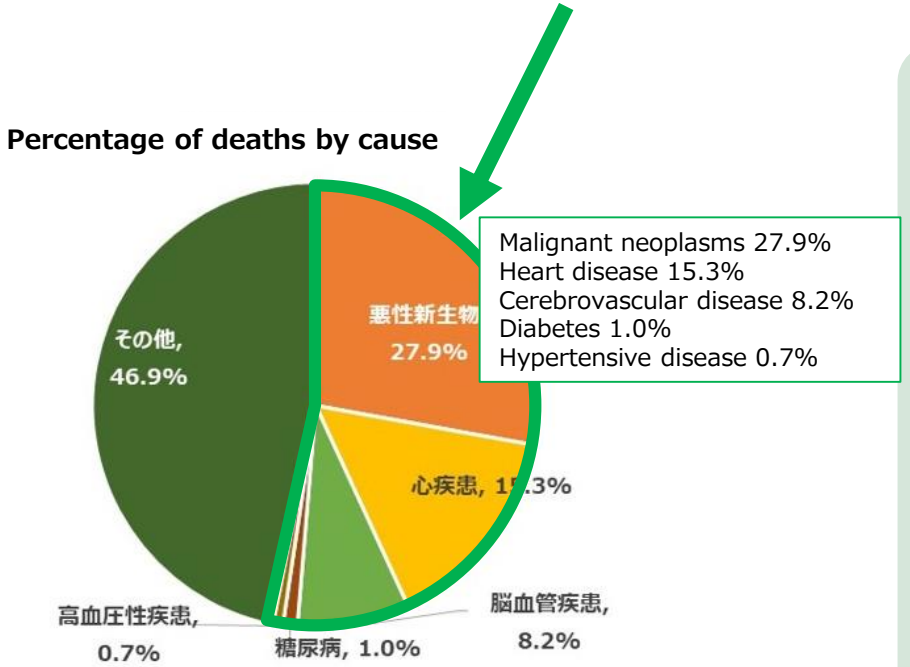
■Lifestyle diseases account for about 30% of medical expenses and about 50% of deaths

Composition ratio of general medical expenses



Source: "Overview of National Medical Expenditures in FY 2016" Ministry of Health, Labour and Welfare

Percentage of deaths by cause



Source: "Overview of Vital Statistics (Final Figures) for 2017" Ministry of Health, Labour and Welfare

Excessive fat storage

↓

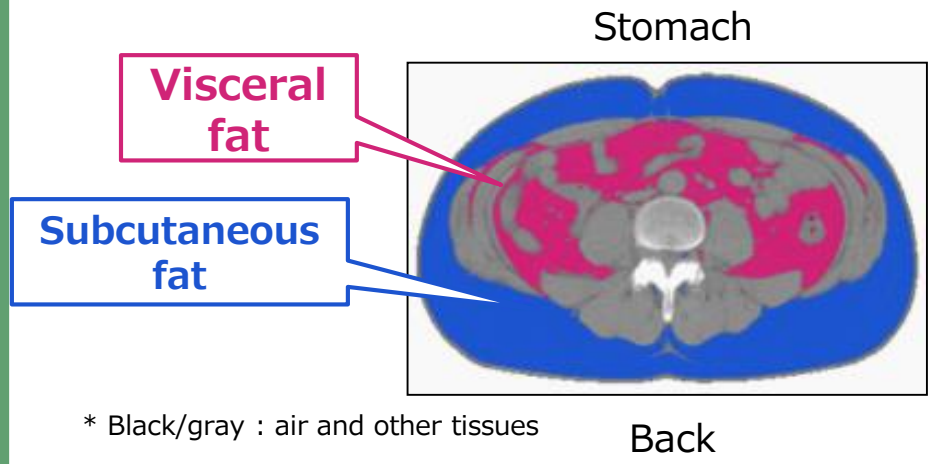
Increase risk of disease

- Diabetes
- Arteriosclerosis
- Fatty liver

About this technology

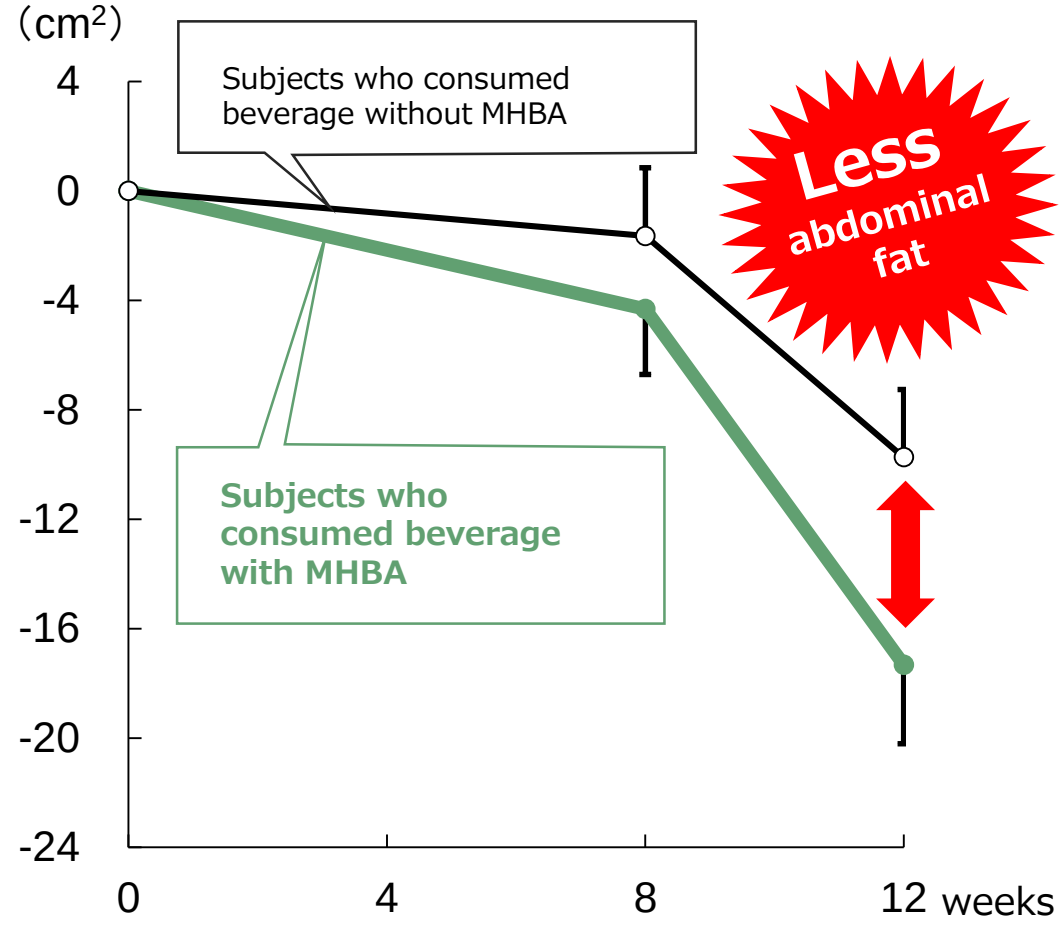
Matured hop bitter acids (MHBA) is an ingredient unique to Kirin products that helps reduce body fat

Abdominal fat around the abdomen
= visceral fat + subcutaneous fat



* Black/gray : air and other tissues

Change in total abdominal fat area



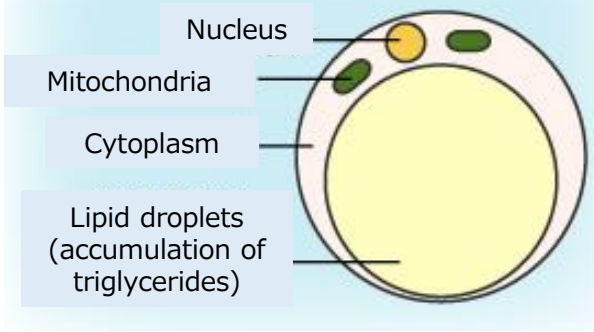
*Morimoto-Kobayashi Y. et al. Nutri J, 2016, 15, 25.
*Yamazaki T. et al. Jpn Pharmacol Ther, 2016, 44, 8. 1193.
*Koizumi K. et al. Jpn Pharmacol Ther, 2016, 44, 8, 1179.

Unique mechanism that MHBA promotes fat burning and thermogenesis

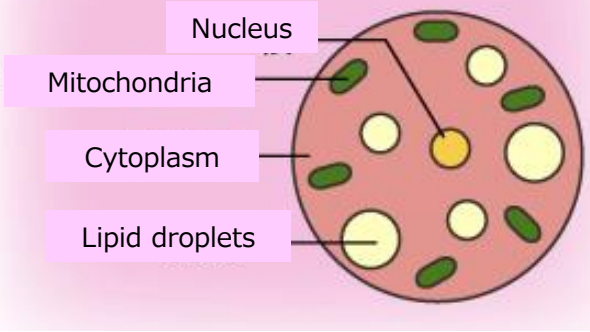
Promotes fat burning and thermogenesis as opposed to suppressing fat absorption or promoting fat breakdown

Two types of adipose cells

Accumulating
white adipose cells



Burning
brown adipose cells



MHBA activates brown adipose cells!

↓

Promotes fat burning!

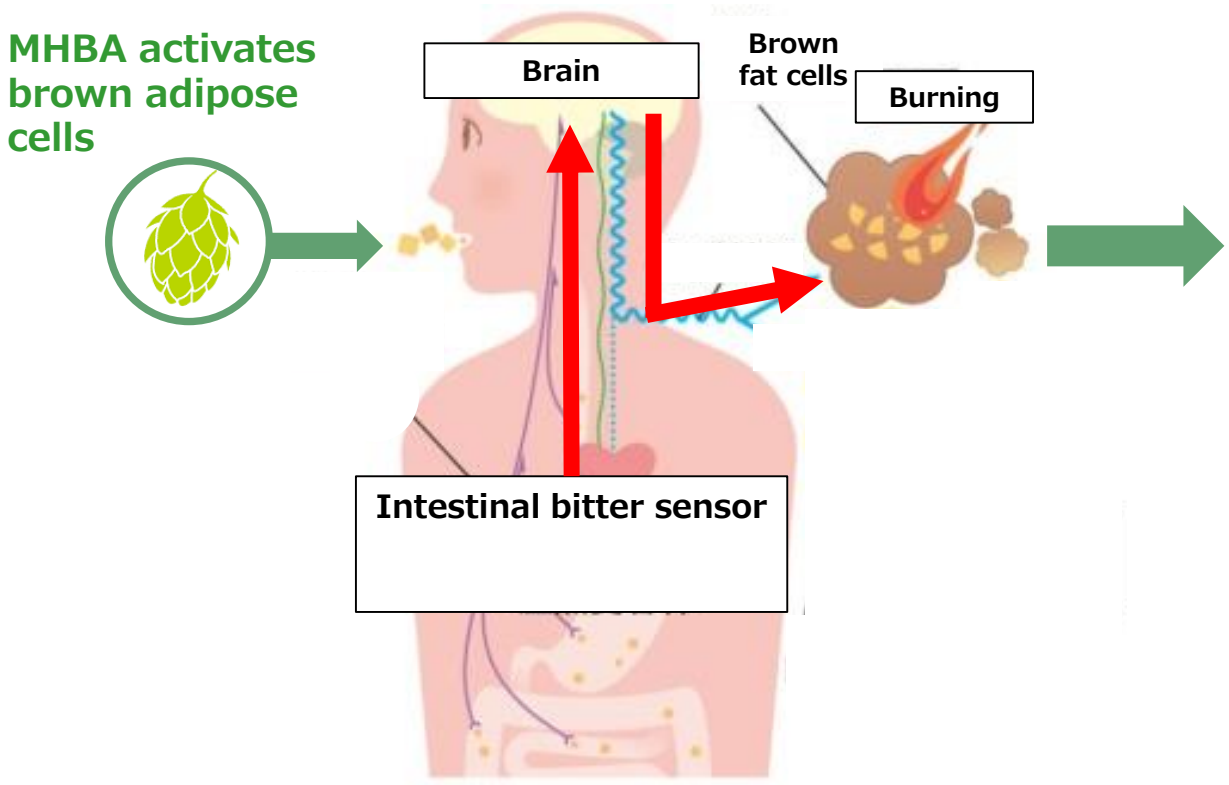
↓

Released as thermal energy!

*Morimoto-kobayashi Y. et al. PloS one, 2015, 10, 6: e0131042.
*Yamazaki T. et al. J Nutr Biochem, 2019, 64, 80.
*Yamazaki T. et al. Biochem Biophys Res Commun, In Submission

About this technology

MHBA is recognized by intestinal bitter sensors, transducing the signal by the gut-brain-brown adipose cell axis, resulting in burning fat.



Body fat reduction effect

Unlike many other food ingredients, MHBA works without being absorbed by the body !

*Morimoto-kobayashi Y. et al. PloS one, 2015, 10, 6: e0131042.
*Yamazaki T. et al. J Nutr Biochem, 2019, 64, 80.
*Yamazaki T. et al. Biochem Biophys Res Commun, In Submission.

History of this technology's development

Kirin's new and unique matured hop extract, which works to reduce body fat without strong bitterness, was developed by taking a "reverse thinking" approach to aging

Ingredients in hops

Alpha-acid

(bitterness component of hops)

Deliberately age the hops to break down the alpha acids !

Brewing beer with fresh hops

Ingredients in beer

Iso-alpha acids

Can reduce body fat, but is too bitter to the taste…

Ingredients in aged hops

Matured hop bitter acids (MHBA)

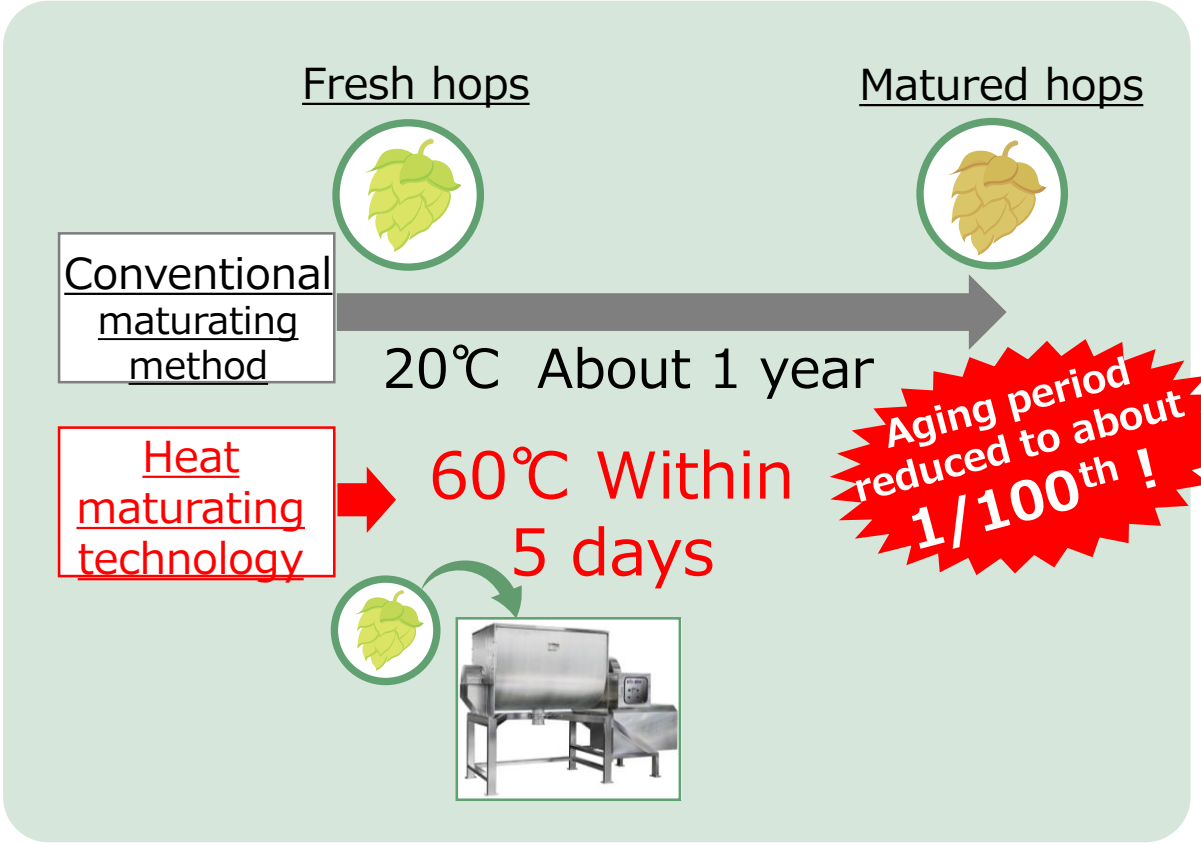
Extract matured hops with water

Matured hop extract

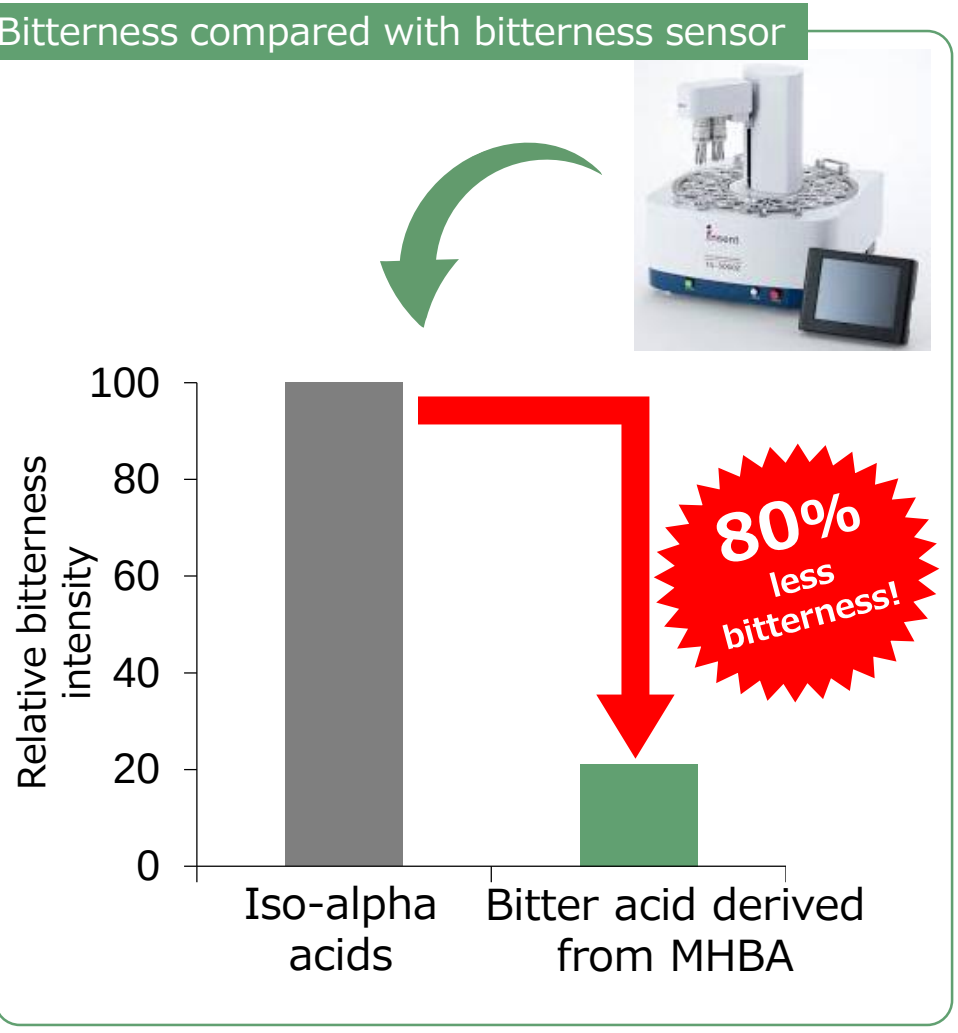
(Extract containing MHBA))

History of this technology's development

" Heat maturating " technology enabling the quick maturation of hops was developed over the course of eight years



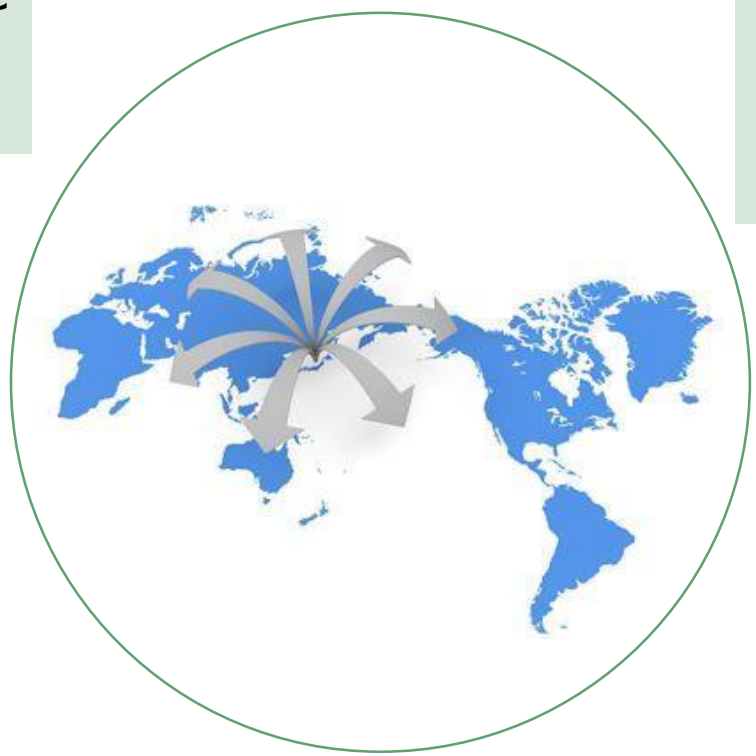
Bitterness compared with bitterness sensor



*Taniguchi Y. et al. Biosci Biotechnol Biochem, 2015, 79.10: 1684-1694.
*Taniguchi Y. et al. J Agric Food Chemistry, 2013, 61.12: 3121-3130.
*Taniguchi Y. et al. J Nat Prod, 2014, 77.6: 1252-1261.
*Yamazaki T. et al. 36th European Brewery Convention, 2017.

Liquid/powder form of matured hop extract can be applied to various foods in Japan and overseas.

Matured hop extract can be manufactured in either liquid or powder form



Has potential for extensive use in beverages, supplements, confectionery and other products

Uniqueness of this technique

Unique material derived from beer ingredients thanks to Kirin's technological capabilities

Characteristic flavor with reduced bitterness

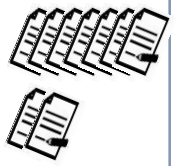
Natural material derived from hops

Highly reliable evidence

Extensive application potential

Abundant evidence

- 7 research papers on effectiveness
- 2 research papers on safety

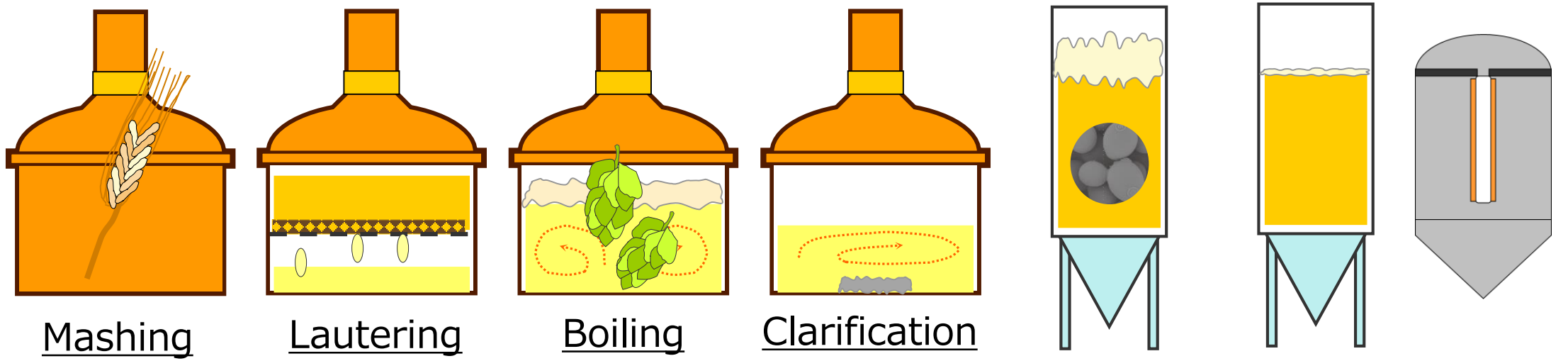


Highly rated both in Japan and abroad

Robust patent network already in place

Beer brewing involves numerous steps and takes more than 1 month

The process of wort production	The process of beer production



Challenges of beer product development

When developing a beer product, a process of trial and error is repeated that consists of designing brewing conditions to match the desired taste, carrying out test brews and evaluating the results

Product planning | Content development | Production

Test brewing

- Raw materials used
- Fermentation conditions …

Brewing condition design

Brewing expertise

- Bitterness
- Aroma
- Mouthfeel …

Evaluation/interview

Challenges of beer product development

Repeating test brews multiple times over is a lengthy process, and design requires the developers' individual experience and intuition

Product planning | Content development | Production

Developer's individual capabilities
Experience/ideas/ knowledge

Test brewing

About one month per cycle

Brewing condition design

Evaluation/interview

About this technology “Takumi” AI

Developed an AI-based system that predicts test results in advance

Product planning | Content development | Production

Test brewing

“Takumi” AI

Brewing condition design

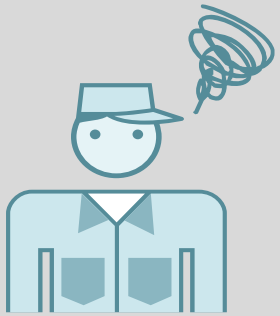
Evaluation/interview

Predicts results without testing!

What can be expected with this technology

The combination of human and AI inputs will shorten development times while helping us gather highly accurate expertise data and pass on technologies in order to deal with increasingly diversified preferences promptly

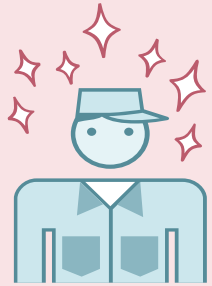
Before（development by humans alone）



Differences in individual skills

Vast numbers of tests repeated

After（development involving cooperation and division of roles between humans and AI）



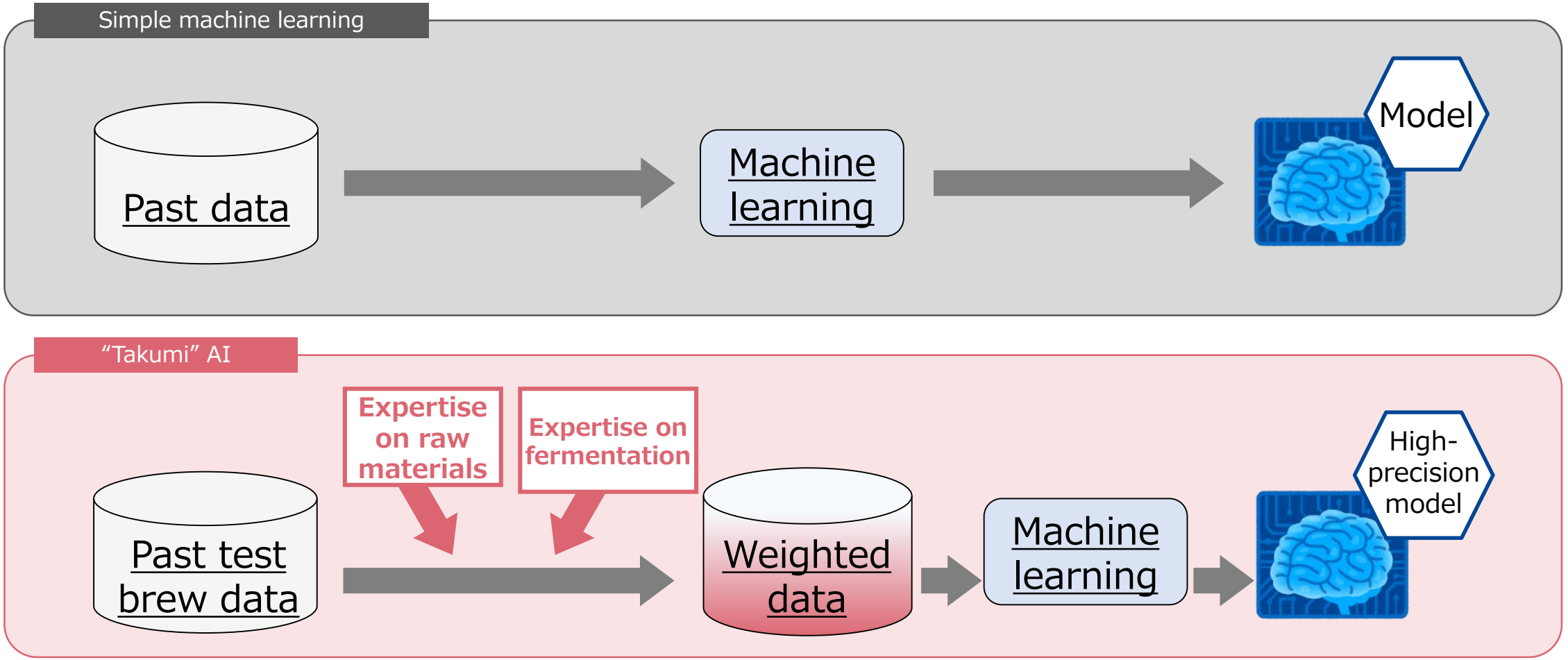
Expertise data collection

“Takumi” AI

Numbers of tests reduced

Features of this technology

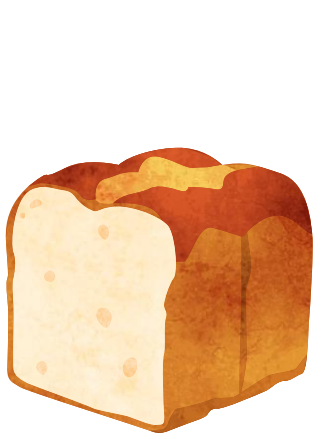
We have created a highly accurate model by combining beer brewing expertise with data science, and have applied to have this patented



Fermentation is the transformation of sugar and other nutrients into something that is beneficial to humans through the action of microorganisms

Food and alcohol

Yeast



Lactic-acid bacilli

Bacillus natto (fermented soybeans)

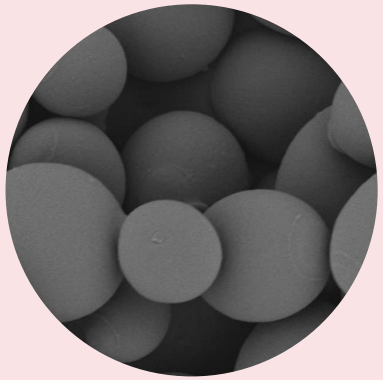
Raw materials for foodstuff and pharmaceuticals

Amino acid-producing bacteria

Beer is made by alcoholic fermentation with brewer's yeast

Malt-derived

- Glucose
- Maltose



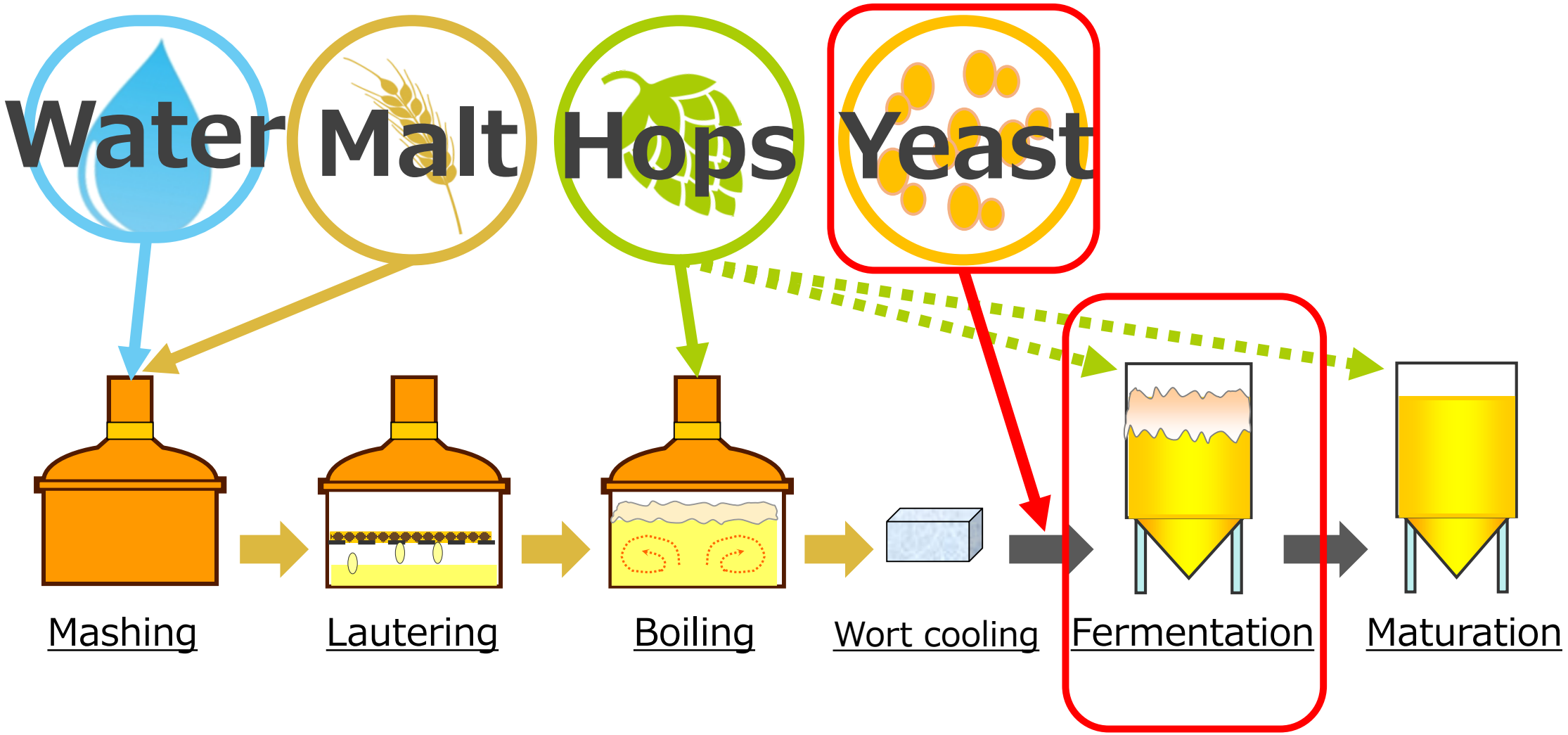
- Brewer's yeast

Alcoholic fermentation

Beer

- Alcohol
- Carbon dioxide
- Flavoring ingredients

Fermentation in beer

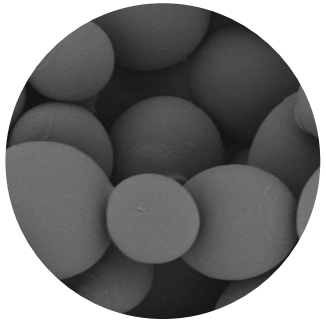


Fermentation in beer

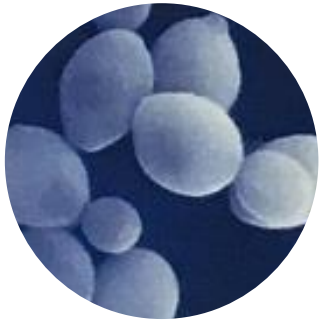
The flavor of the beer will vary depending on the type of brewer's yeast and fermentation conditions

Main yeast types

Lager yeast



Ale yeast



Lager beer

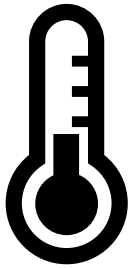
•Refreshing
and easy-to-drink

Ale beer

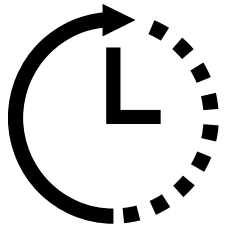
•Rich taste

Fermentation conditions

Temperature



Time



Aroma

Taste

Smoothness going down

etc...

We have produced various types of yeast using our unique technology Currently, we have about 1,000 varieties of brewer's yeast at our disposal

Brewer's yeast bank

Creation of yeast with new characteristics

- Natural mutation
- Crossing of different yeast

Enhancing our brewer's yeast bank

Selection

Beers are developed from various yeasts

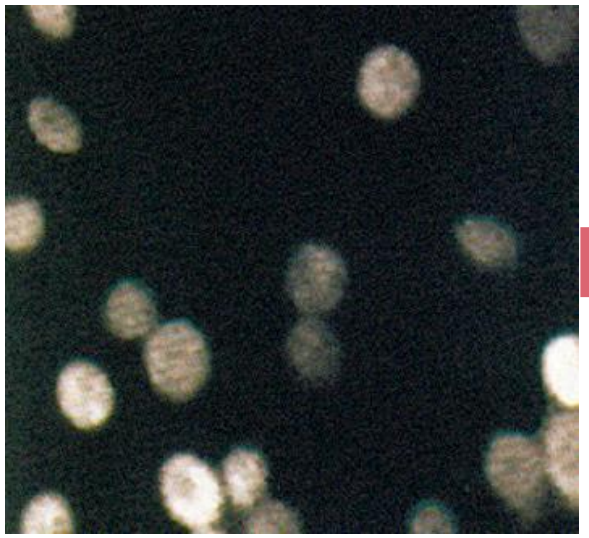
Kirin's unique technologies (2)

We have the technology to visualize and appropriately control the "health status" of brewer's yeast, which affects the taste of beer

Healthy yeast

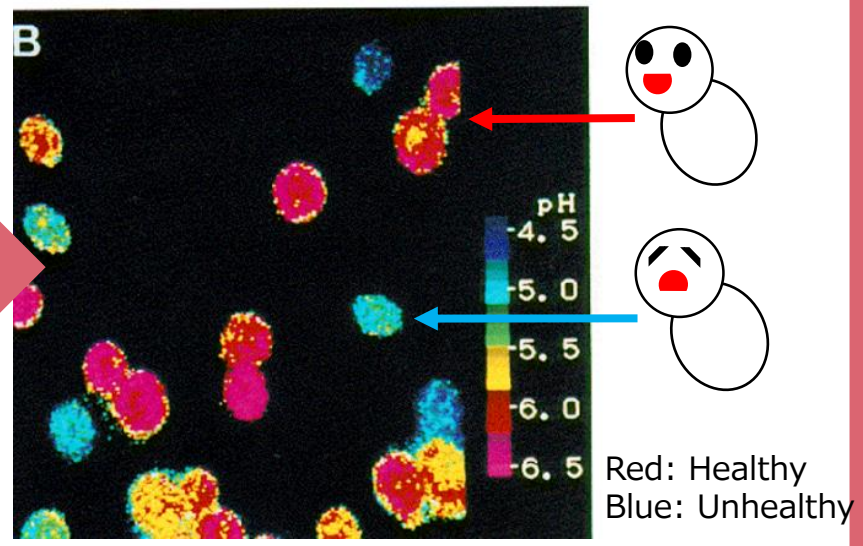
Unhealthy yeast

Cannot be identified with a normal microscope



Color-coded by pH, which serves as an indicator of yeast health

Health status can be distinguished based on color !

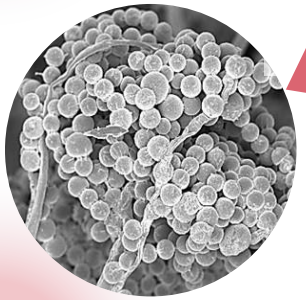


Red: Healthy
Blue: Unhealthy

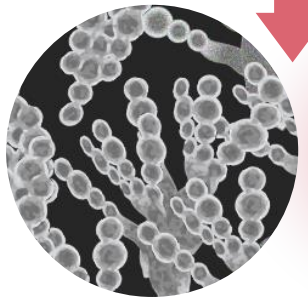
Application of the technology to other microorganisms

We have expanded our yeast technology by applying it to other microorganisms

Lactic acid bacteria

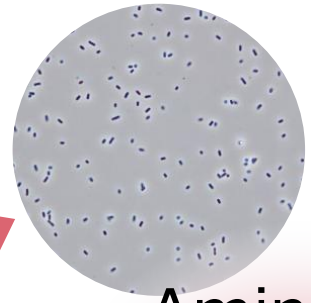


Asrergillus

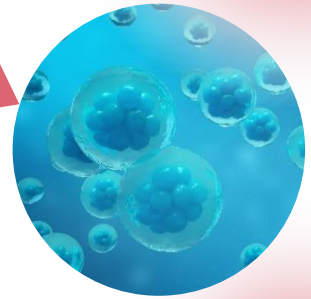


Penicillium camemberti

Yeast control technology <Fermentation /biotechnology>



Amino acid producing bacteria
⇒Kyowa Hakko Bio

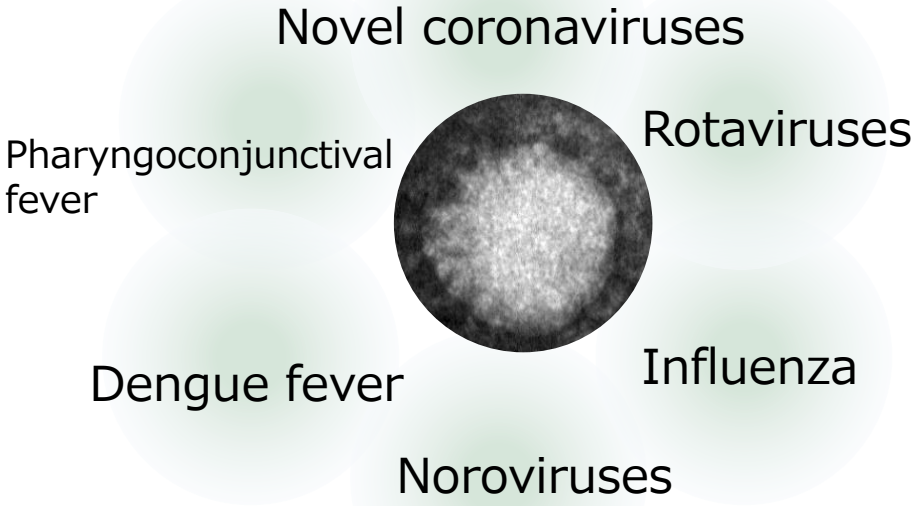


Animal cells
⇒Kyowa Kirin

What can be expected from this technology

Control infectious disease risk and solve the problem of infectious disease in areas with poor sanitary conditions

Threat of viruses throughout the year
Risk of new viruses emerging



Pandemics caused by the movement of people and goods

Limitations of medical treatment and vaccines

It is increasingly important to strengthen the body's natural immunity to fight viruses in the course of daily life.

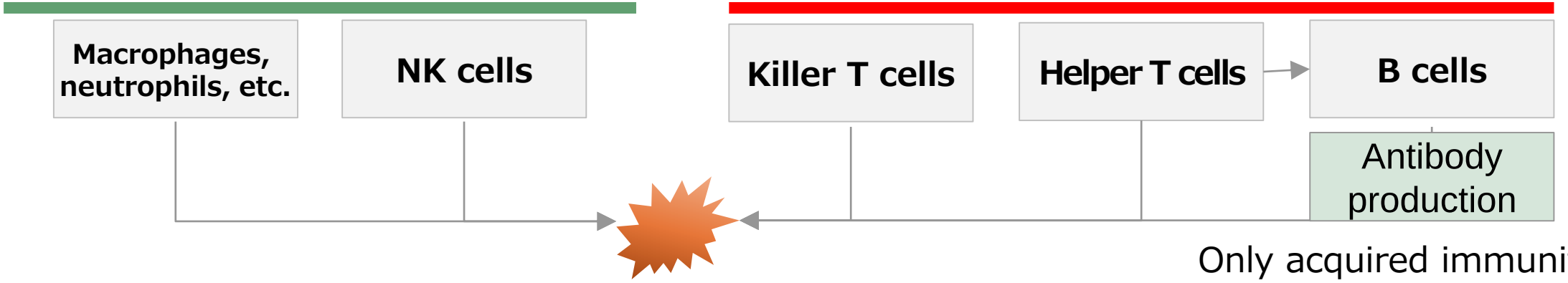
A mechanism for eliminating viruses, bacteria and other pathogens through both innate and acquired immunity

First type of immunity: innate immunity

- An immune response mechanism that people are born with
- Relays information on the enemy to acquired immune cells
- Its offensive power is weak, but its response is immediate (several hours)
- The innate immune system does not retain any memory of its targets, and simply attacks the enemy at hand

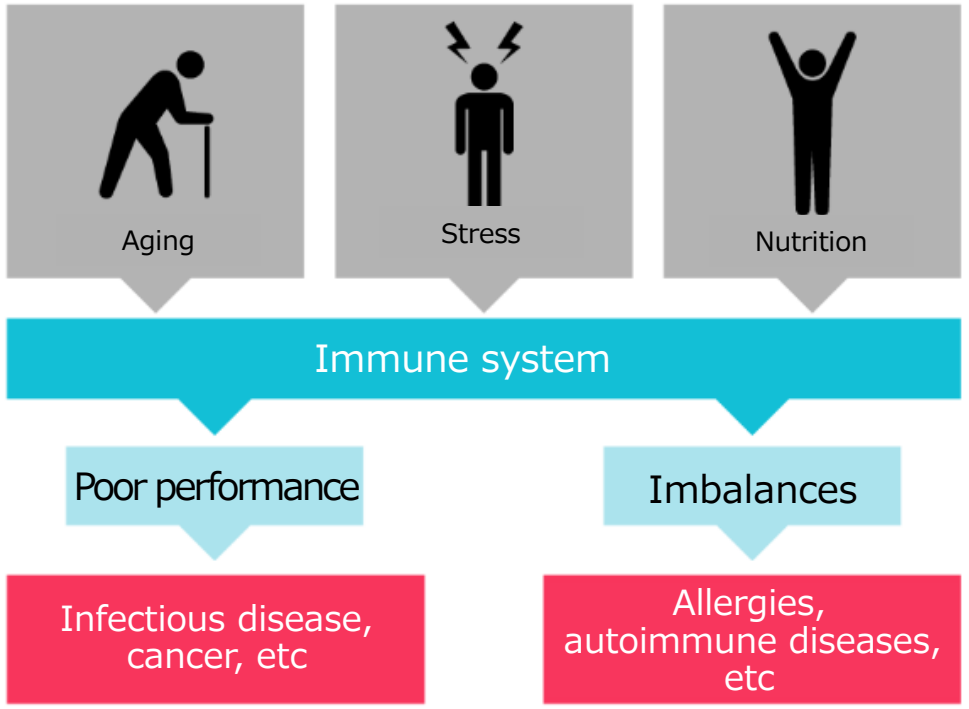
Second type of immunity: acquired immunity

- Acquired immune response
- Attacks enemies that breach innate immunity
- Is powerful but takes a few days to kick in
- Retains memory of the target (response is immediate from the second time onwards)

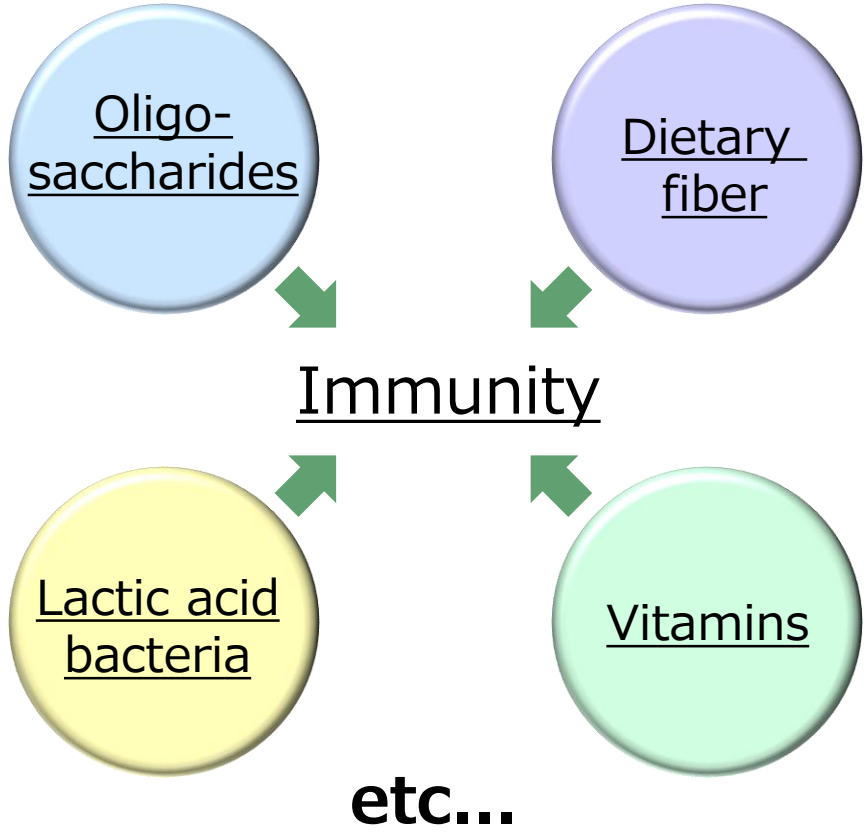


Dietary habits greatly affect immunity
Lactic acid bacteria are known to be closely linked to immunity

Relationship between immunity and daily life



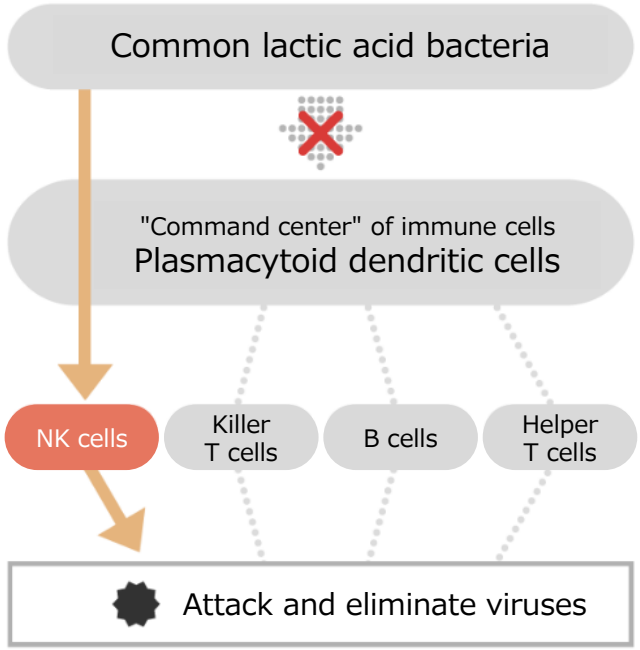
Food ingredients known to be associated with immunity



Background of this technique

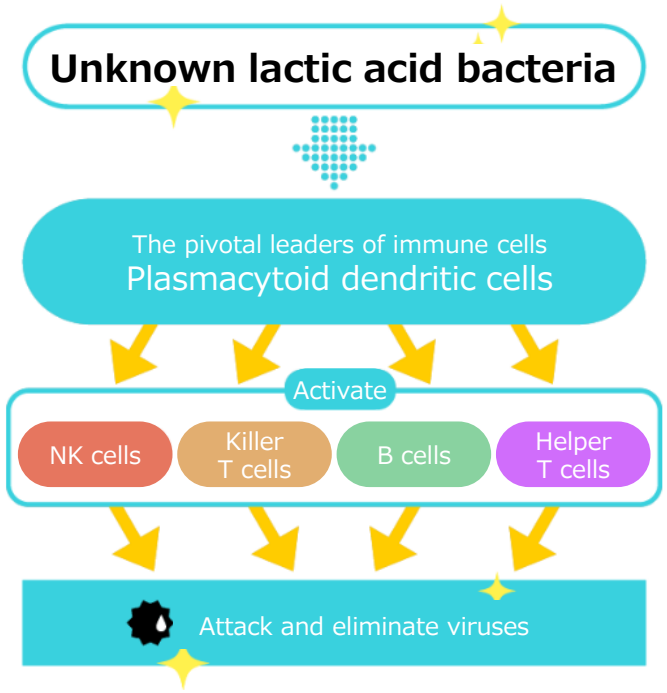
In the past, the accepted knowledge in immunology was that lactic acid bacteria activate only some immune cells (NK cells).

Accepted knowledge in immunology



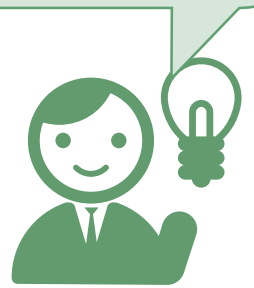
Activate only some cells

Kirin's idea



Activates all immune cells

Is it possible that there may be lactic acid bacteria capable of activating the control tower?

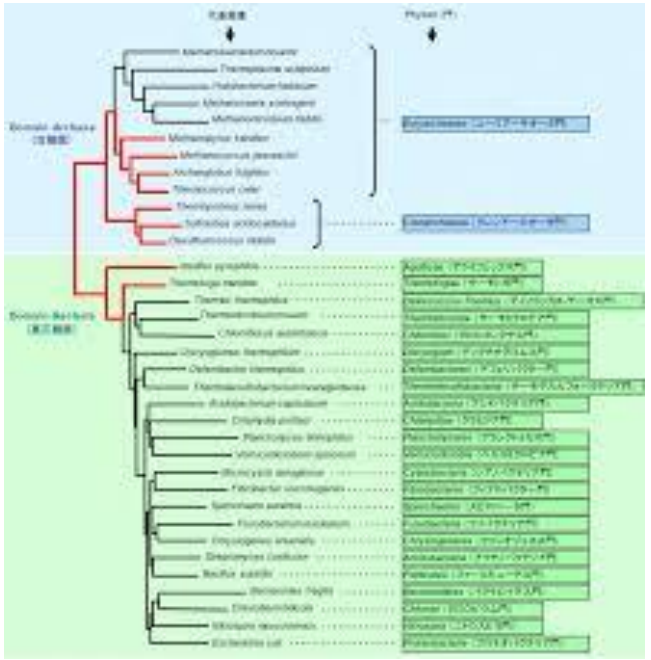


*Blood 2009;113:4232-4239. Human plasmacytoid dendritic cells are unresponsive to bacterial stimulation and require a novel type of cooperation with myeloid dendritic cells for maturation

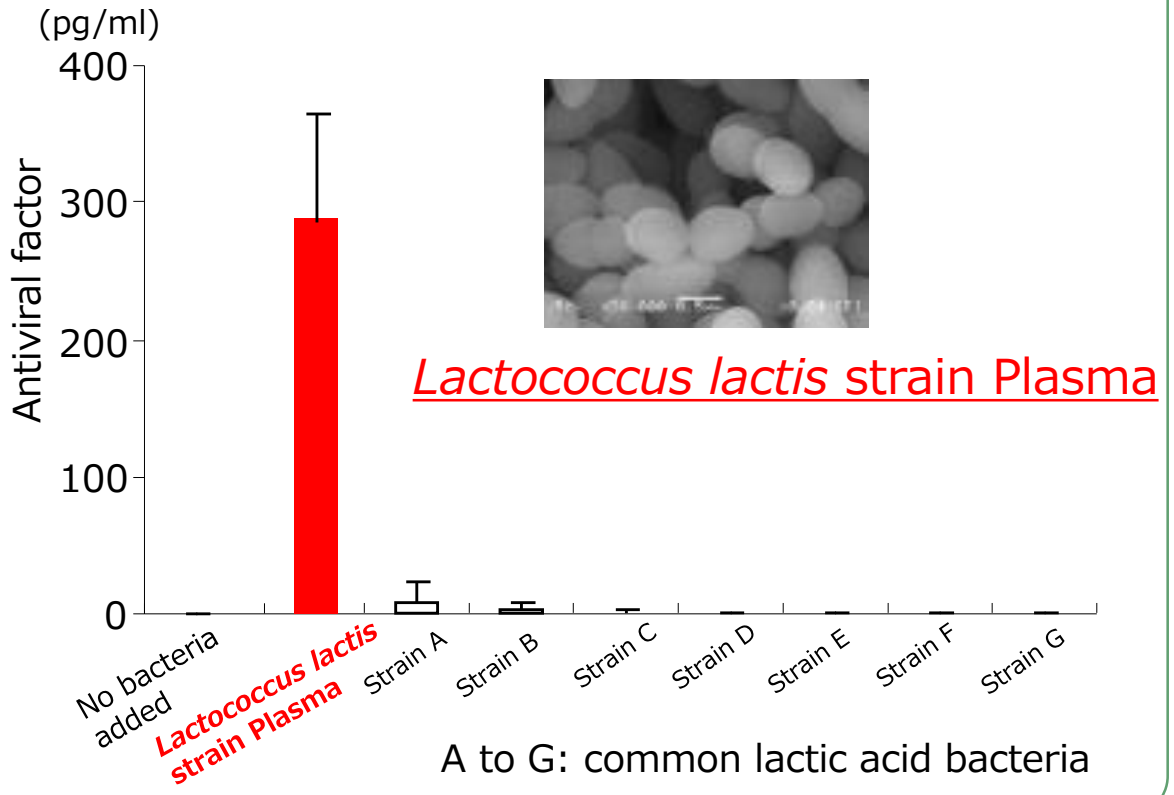
About this technology

By questioning accepted knowledge and studying large numbers of lactic acid bacteria, we discovered "*Lactococcus lactis* strain Plasma" — a type of bacteria that activates the control tower

Studied large numbers of lactic acid bacteria in the microbial strain bank



Degree of activation of pDC



*Presentation: The 48th Annual Meeting of the Japanese Society for Pediatric Infectious Diseases

About this technology

Ingestion of "*Lactococcus lactis* strain Plasma " reduced the risk of influenza and colds

We asked 200 people to consume either a milk-based drink containing "*Lactococcus lactis* strain Plasma" or a milk-based drink without "*Lactococcus lactis* strain Plasma" every day for 10 weeks, and investigated the effects on their physical condition.

Volunteers

200people

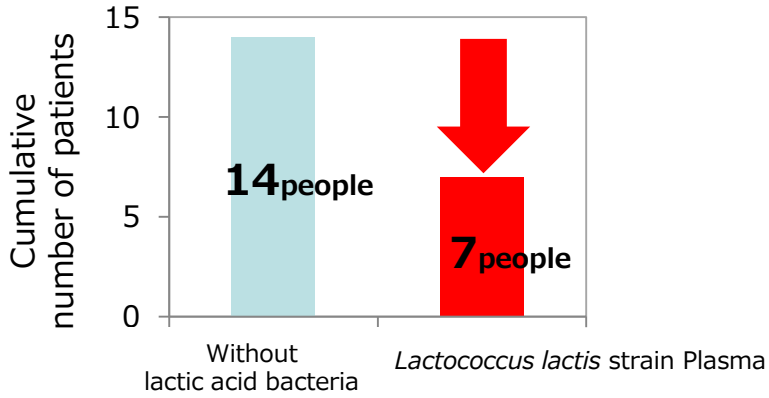
100people

Milk-based drink containing *Lactococcus lactis* strain Plasma

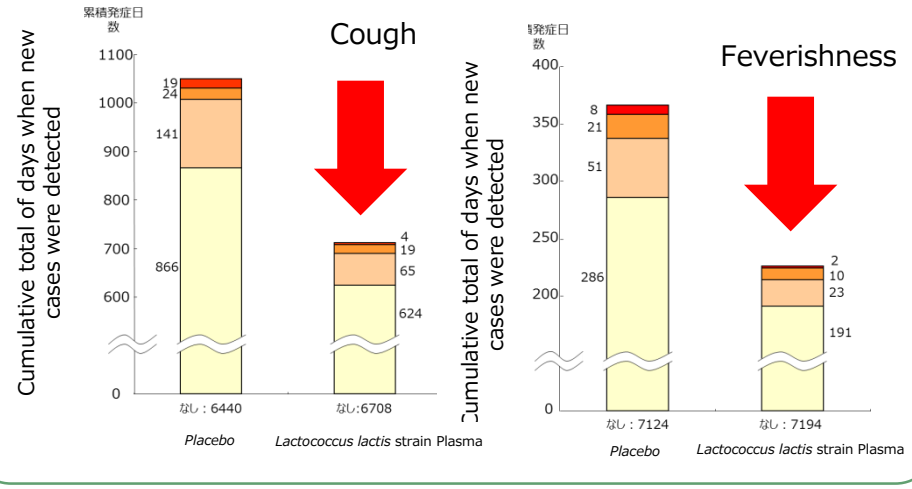
100people

Milk-based drink that does not contain *Lactococcus lactis* strain Plasma

Number of people suffering from influenza/cold

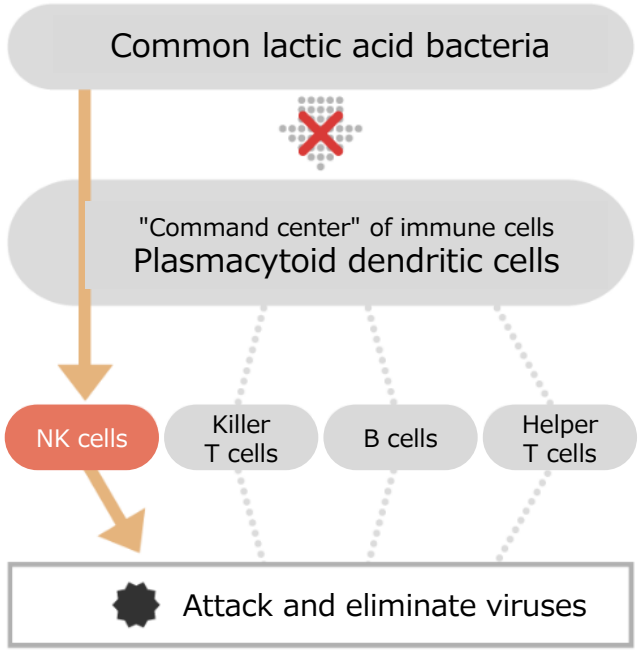


Influenza-like subjective symptoms

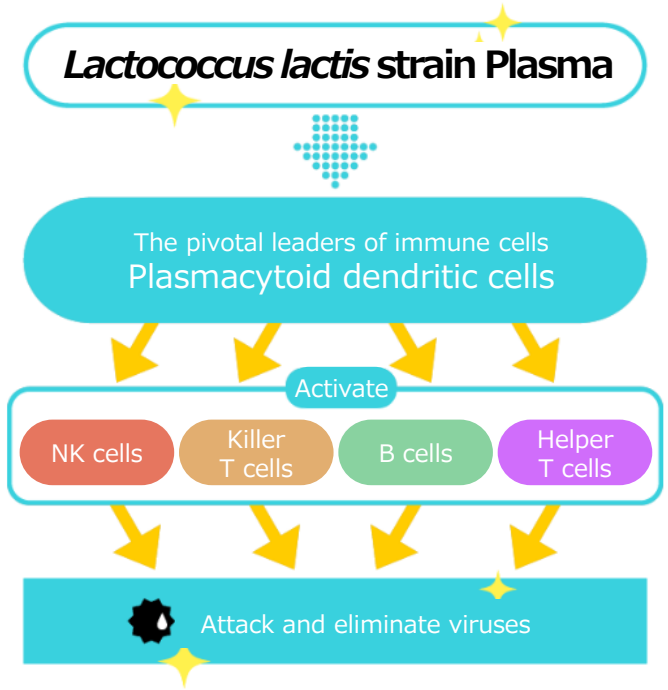


Uniqueness of this technique

Lactococcus lactis strain Plasma are highly rated by experts for their unique ability to activate the "control tower," with numerous scientific papers published



Activate only some cells



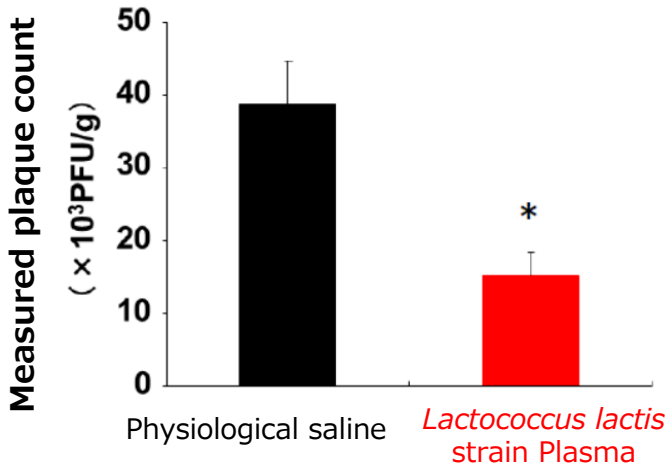
Activates all immune cells

32 papers published in total!

Future potential

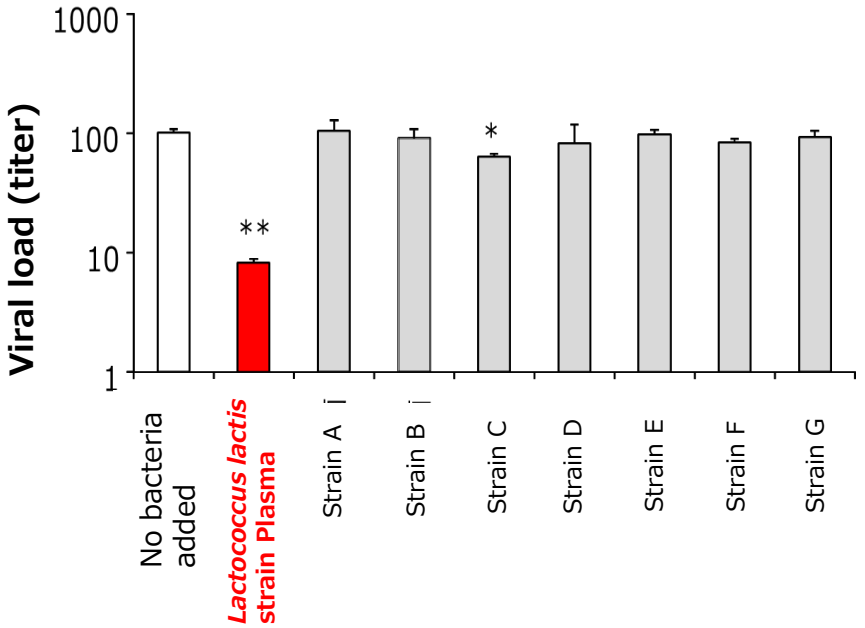
Since *Lactococcus lactis* strain Plasma activate the "control tower," they can be expected to be effective against various viruses

Effect on rotavirus (non-clinical study)



A model of rotavirus infection was used to evaluate the effects of *Lactococcus lactis* strain Plasma. Compared to the physiological saline group, the *Lactococcus lactis* strain Plasma group showed an improvement in fecal rotavirus levels

Effect on rotavirus (non-clinical study)



Dentritic cell supernatant stimulated with Lactococcus lactis strain Plasma was added to cultured cells infected with dengue virus. This was found to limit virus growth

If you would like to find out more:

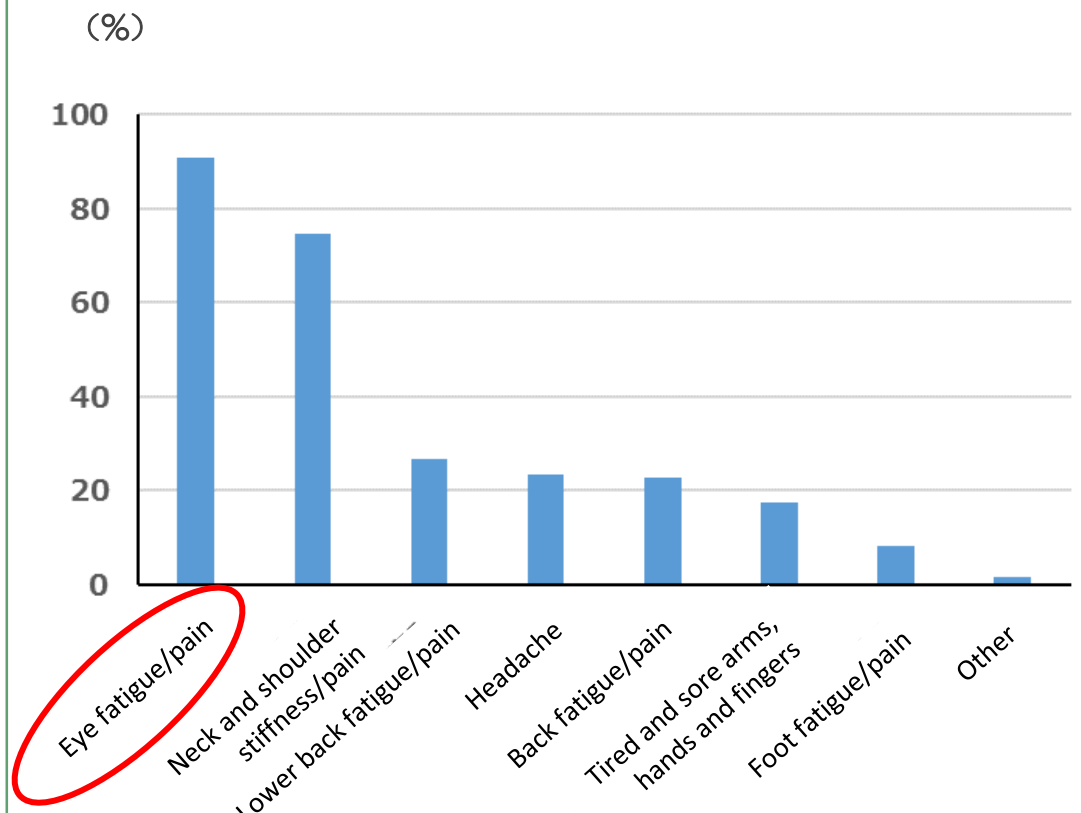
Lactococcus lactis strain Plasma Research Report | Search

Background of this technology

As our society ages and digitalization advances, eye fatigue is becoming an issue

Physical fatigue and symptoms felt due to digital work

Source: Survey on Technological Innovation and Labour 2008 (Ministry of Health, Labour and Welfare)



PC work

Online lessons

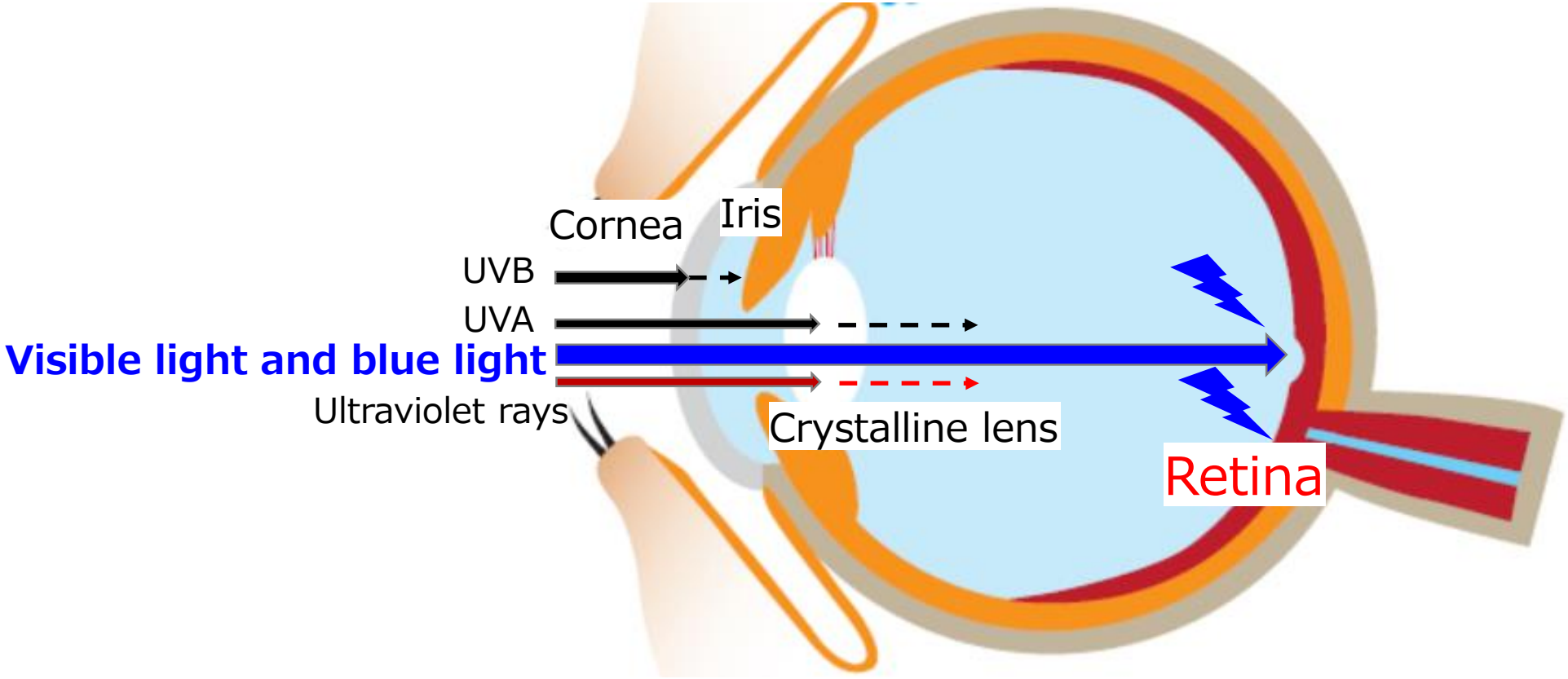
Aging

Smartphones

VR goggles

Visible light and blue light can cause inflammation of the retina

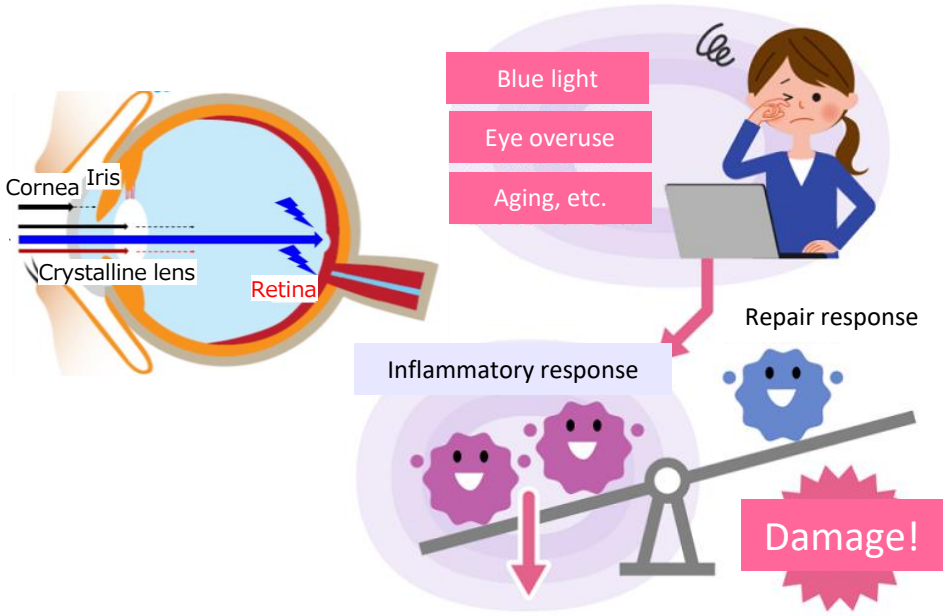
Visible light and blue light reach the retina at the back of the eye. Excessive exposure to these lights damages the retina and triggers an inflammatory response.



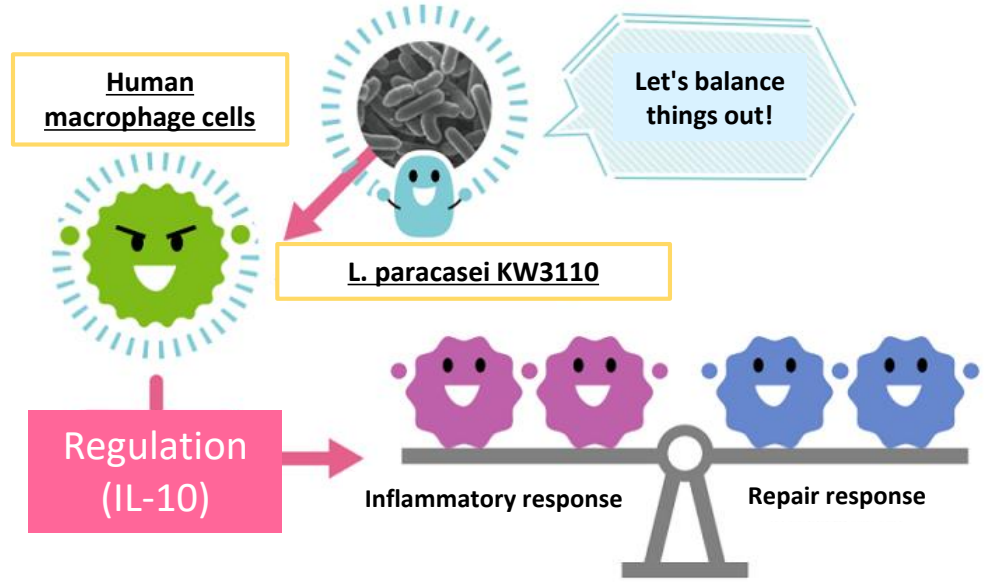
About this technology

KW lactobacilli balance repair and inflammation

Inflammation of retinal cells causes eye fatigue.
KW lactobacilli shift the balance toward repair.



Blue light and other factors can irritate the eyes, in turn resulting in inflammation of retinal cells.



KW lactobacilli (L.paracasei KW3110) act on macrophages* to balance inflammation.

*An immune cell that preys on foreign organisms in the body and secretes various regulators; a type of white blood cell

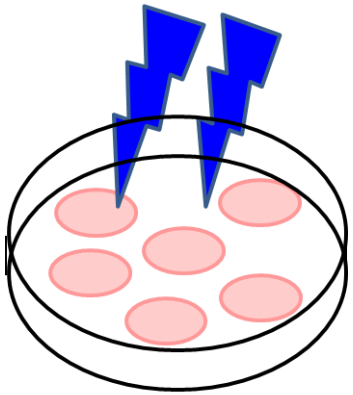
About this technology

KW lactobacilli reduce damage caused to the retina by blue light and aging

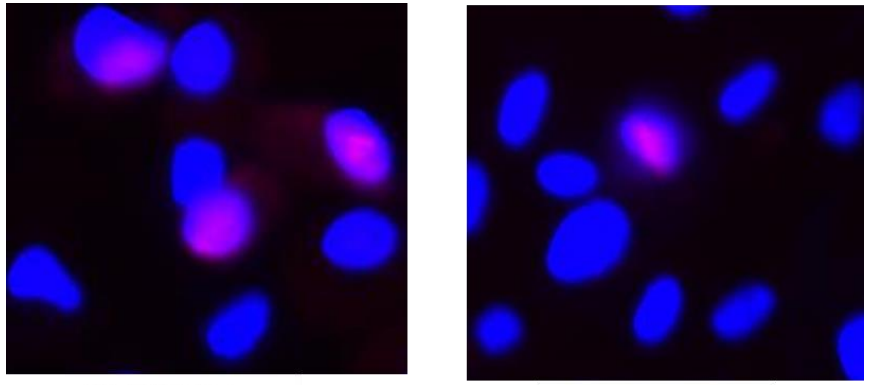
Macrophage cell supernatant stimulated with KW lactobacilli was added to human retinal cell lines. These were then irradiated with blue light, and cell death rates were evaluated.

Human retinal cells　No lactobacilli　KW lactobacilli

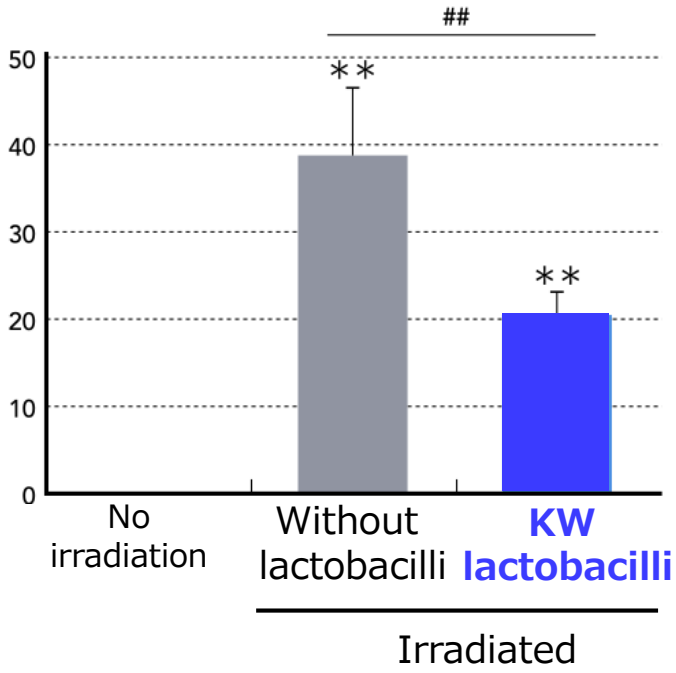
Blue light



Pink: Dead cells



Cell death rate after blue light irradiation



Cell death rate suppressed !

About this technology

Ingestion of KW lactobacilli improves eye fatigue after digital tasks

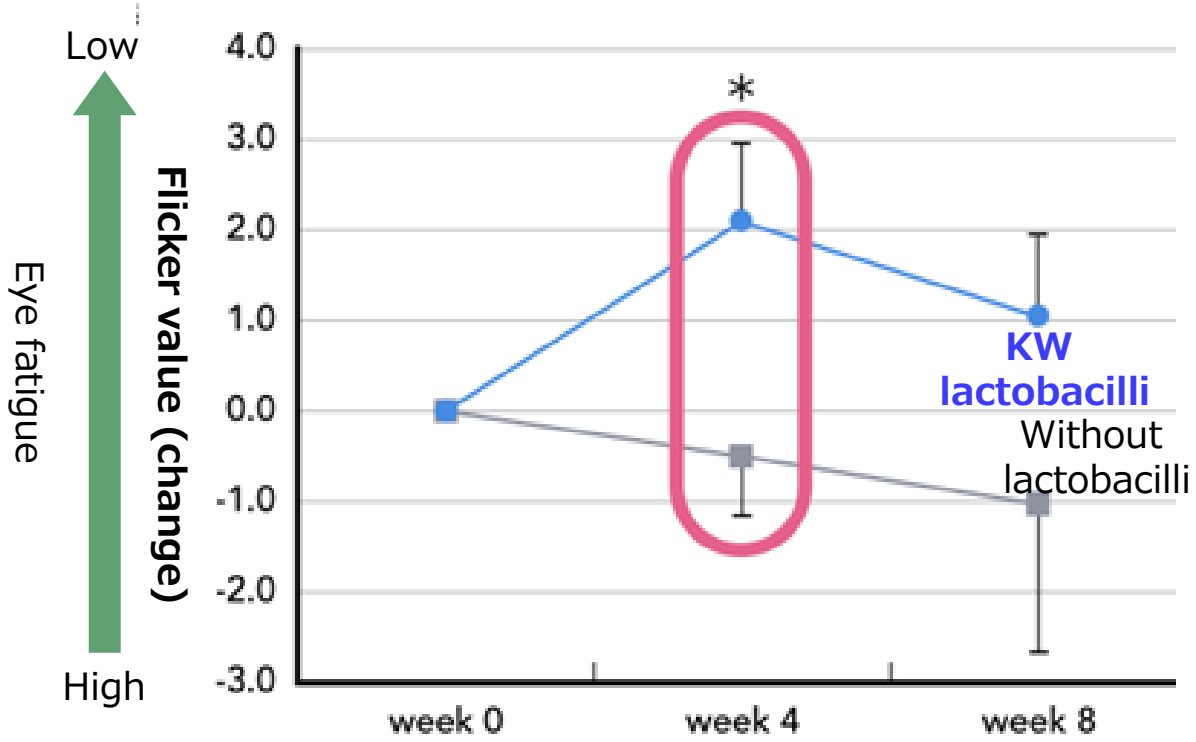
25 people suffering from eye fatigue were asked to take capsules that contained or did not contain KW lactobacilli every day for eight weeks, and to perform digital tasks. Eye fatigue after work was compared by using flicker values* as an indicator.

*Limit speed at which progressively faster flashing of light remains perceptible.
Fatigue is known to reduce perceptibility.

Flicker measuring instrument ▶

Eye fatigue after digital task

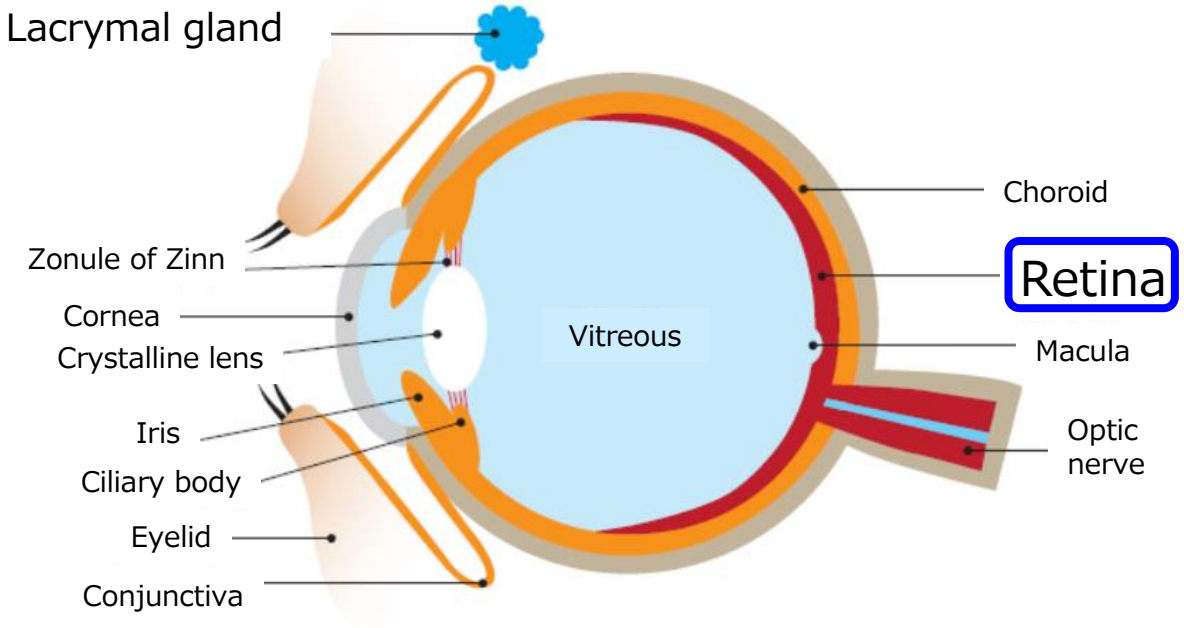
Source : *Nutrients,* 2018



Intake period: 8 weeks

Plasma lactobacillus is the only lactobacillus that has been shown to have a positive effect on the eyes

Unlike conventional approaches, KW lactobacilli are distinguished by their ability to provide support from the inside through their effect on immunity. Only KW lactobacilli have been found to have a positive effect on the eyes



Eye drops → External care → Cornea

Blueberry Lutein → Pigment supplement → Macula Retina

KW lactobacilli → Internal care → Retina

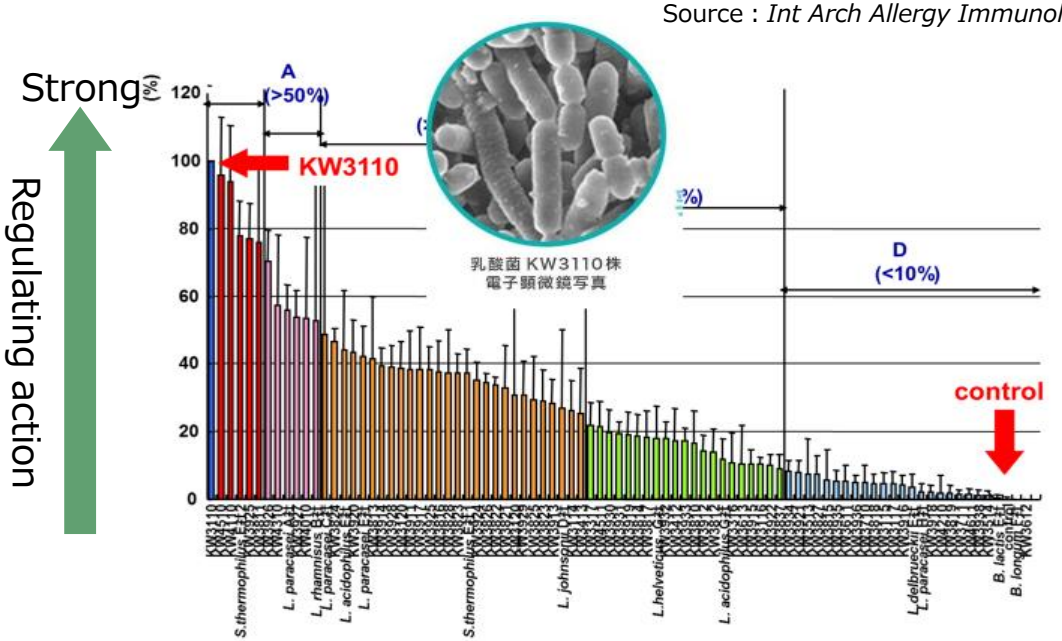
Background of this technology (base technology and its history)

Results of KW lactobacilli research

KW lactobacilli were identified in 2000 in the course of research in the field of immunity, which is one of the Kirin Group's strengths. We have since continued our research on allergy symptoms, and have discovered a novel effect on eye fatigue. Research is continuing in greater depth.

Amount of IL-12 secreted when various lactic acid bacteria are added to immune cells

Source : *Int Arch Allergy Immunol, 2004*



Out of a large number of lactobacilli, KW lactobacilli were found to exhibit the strongest regulating action on the immune imbalance that causes allergies

Benefit for allergic conditions

New function

Benefit for eye fatigue

Potential future applications of KW lactobacilli

■There are many more symptoms involving the eyes in addition to fatigue

■Inflammation can trigger various health conditions

KW lactobacilli

Farsightedness

Age-related macular degeneration

Glaucoma

Bloodshot eyes

Dry eyes

Diabetes

Obesity

Aging

Rough skin

Depression

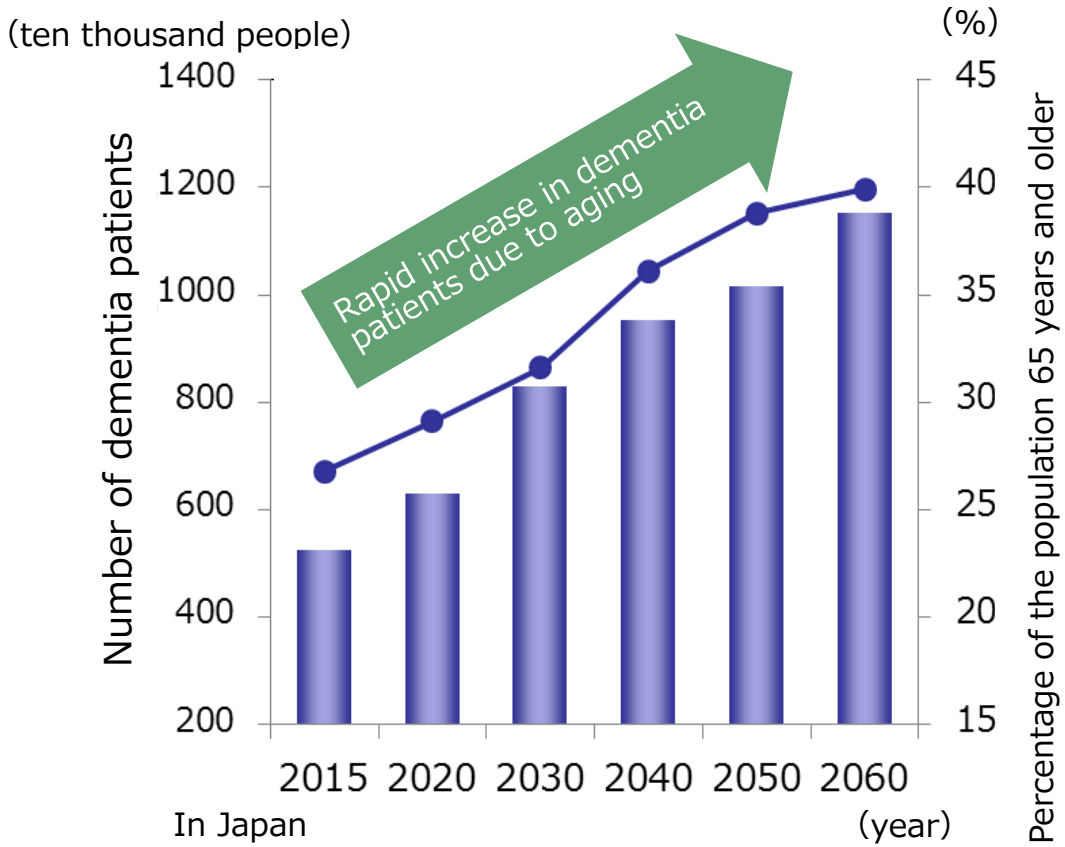
Dementia

→ Looking forward, we hope to expand the range of disorders that can be relieved with KW lactobacilli by leveraging their characteristics — namely, the ability to provide care for the retina from the inside and reduce inflammation

What can be expected from this technology

Using Nutritional approaches to help address the social issue of dementia associated with aging

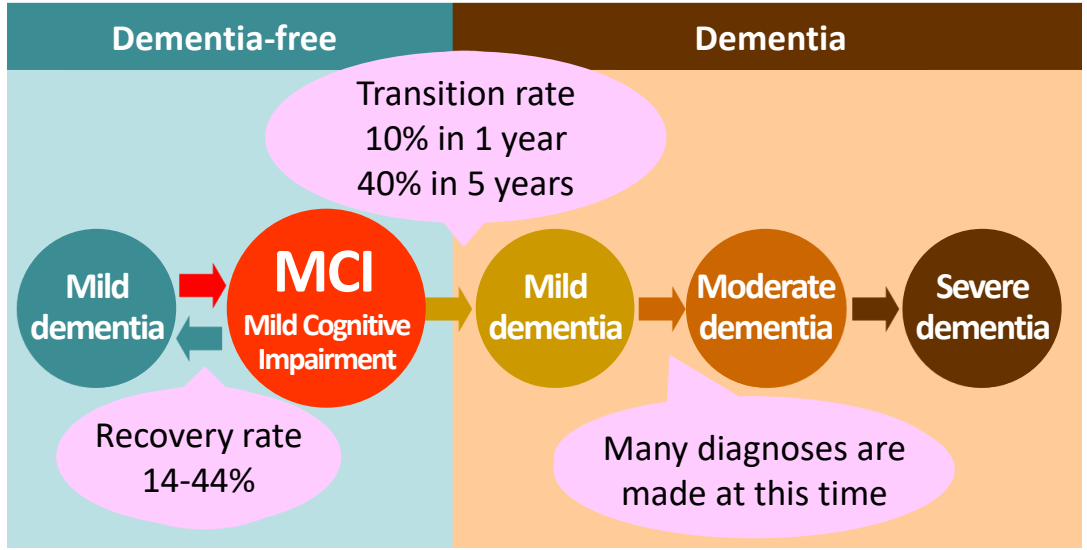
The preservation of brain health is becoming a social issue



Source: Statistics Bureau, Ministry of Internal Affairs and Communications

Appropriate measures for early prevention

Appropriate early measures lead to the preservation of brain health

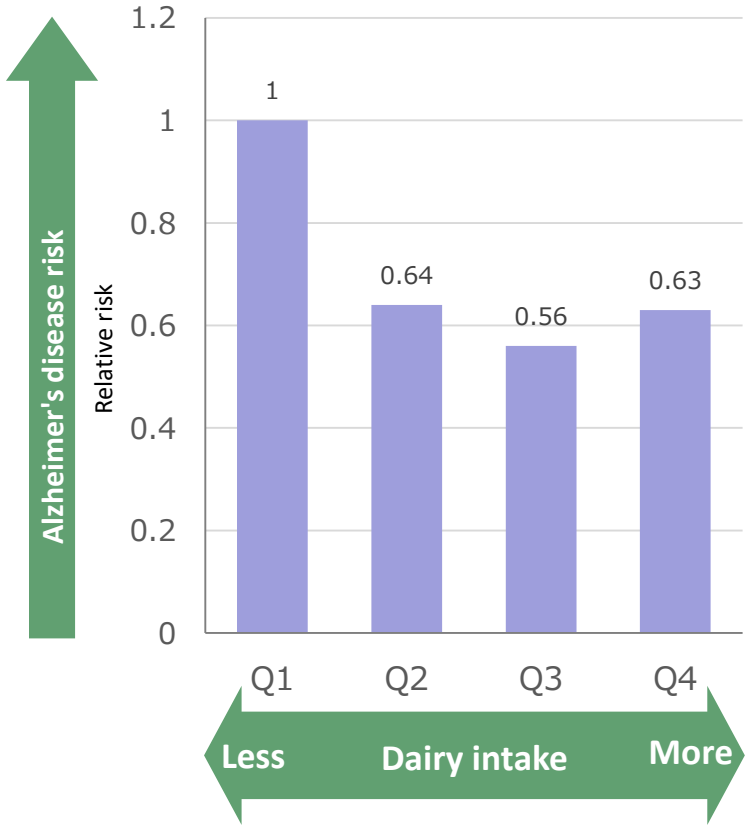


Source: Ninchisho Netto (Dementia Net)

About this technology

Focus on epidemiological reports that dairy intake reduces the risk of dementia

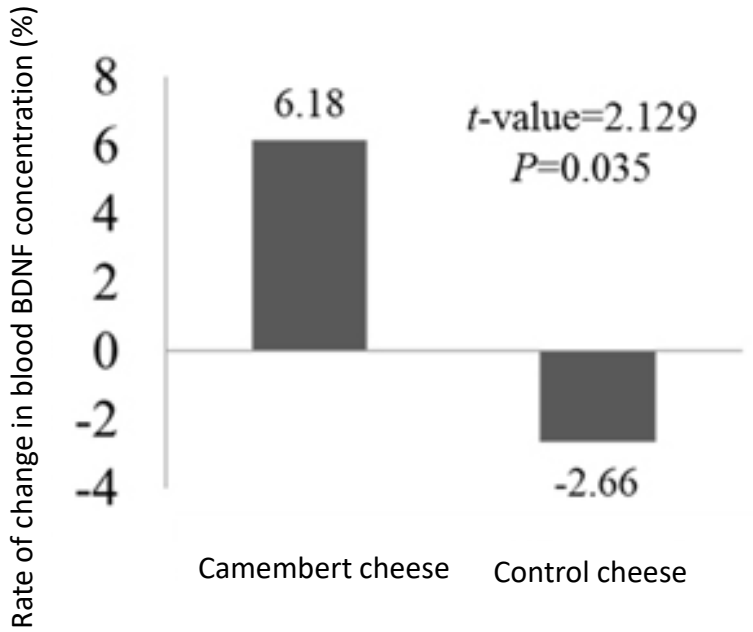
Relationship between consumption habits of fermented dairy products and cognitive function in old age (Hisayama Town Study)



J. Am. Geriatr Soci, 2014

Relationship between continuous consumption of Camembert cheese and blood neurotrophic factors

Clinical trial on MCI and Camembert cheese Blood BDNF results



J. Am. Med. Dir. Assoc., 2019

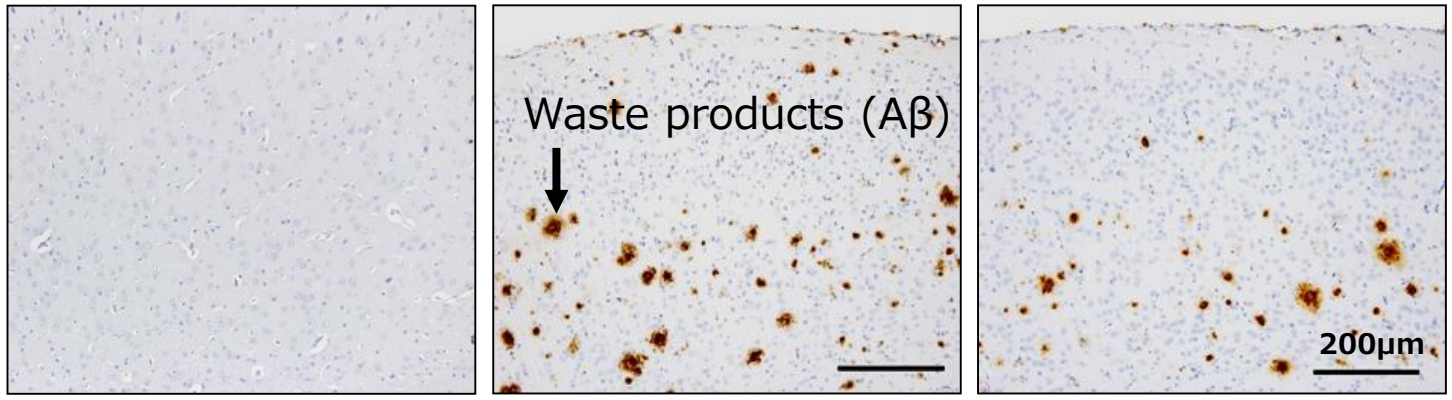
About this technology

The preventive effect of Camembert cheese against dementia was first discovered in collaboration with the University of Tokyo

東京大学
THE UNIVERSITY OF TOKYO

Healthy group | Alzheimer's disease group

Control diet group | Test meal group



Ano et al., PLoS ONE 2015

→ Shed light on the relationship between Camembert cheese consumption and the onset of Alzheimer's disease

About this technology

Camembert cheese can be fermented and aged with white mold and lactic acid bacteria

Milk-based

- Protein
- Fat, etc.

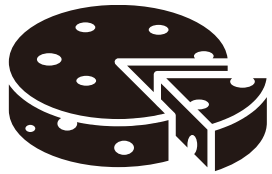
Lactic acid bacteria

White mold

Fermentation & Aging

Ingredients that help improve cognitive function are produced during the fermentation process

Camembert cheese



Milk-based

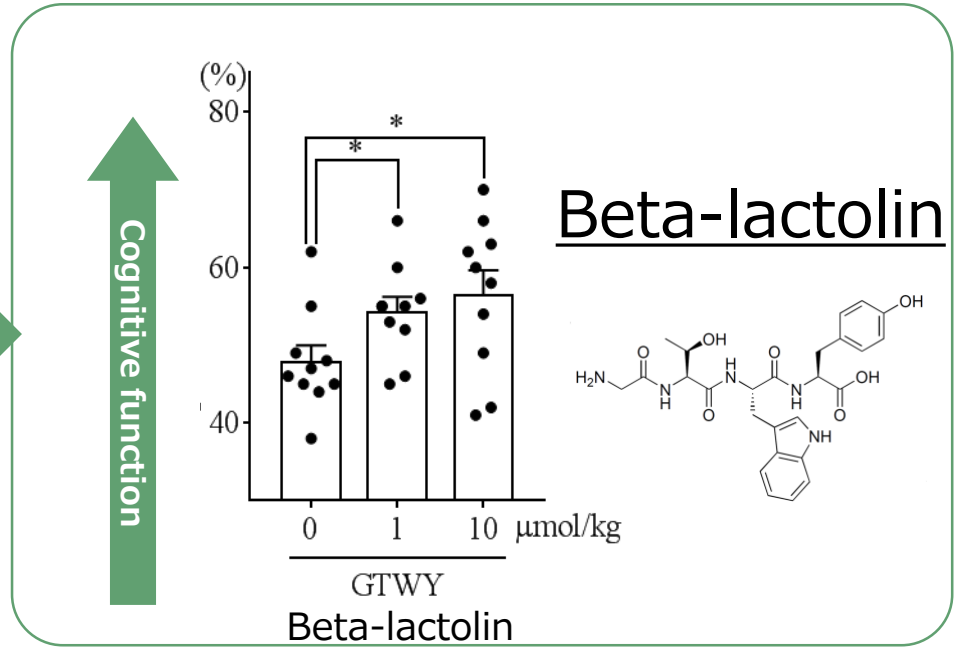
- Peptides
- Fatty acids, etc.

About this technology

We have independently discovered a peptide that can improve cognitive function, and have developed a processing method that facilitates its intake

- We have independently discovered beta-lactolin — an active ingredient in dairy products that improves cognitive function
  We have established a manufacturing method for food ingredients that facilitates beta-lactolin intake

Search



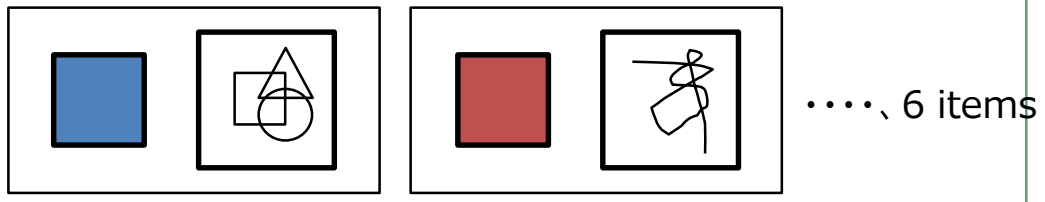
Mass production

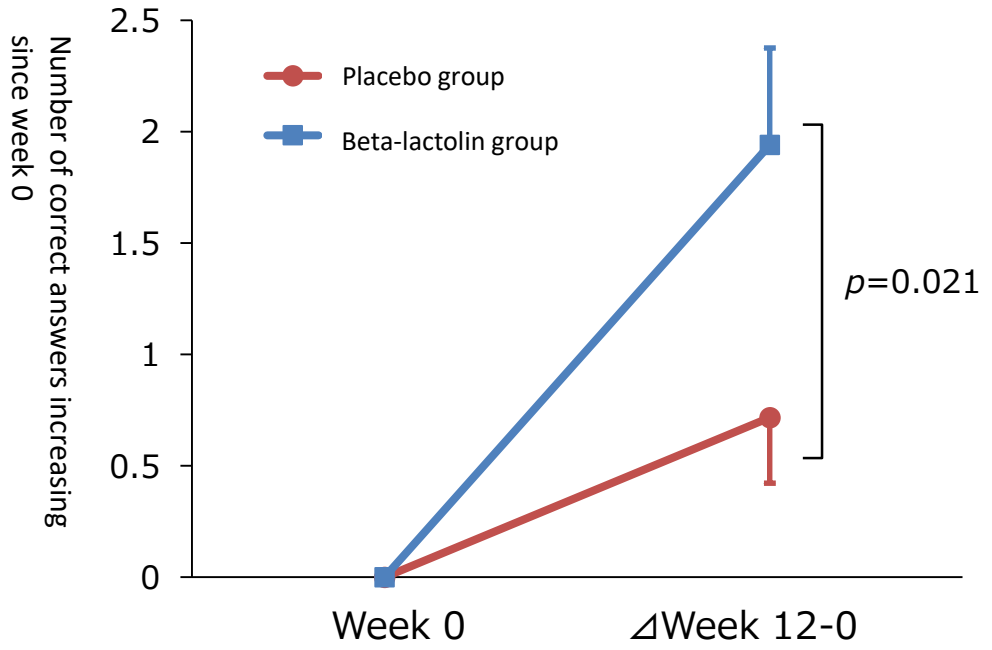
Ano et al., Neurobiol. Aging 2018

About this technology

Improvement in cognitive function (memory/ability to pay attention and concentrate) confirmed in clinical trials

Improved performance in visual paired-associates test (memory recall)

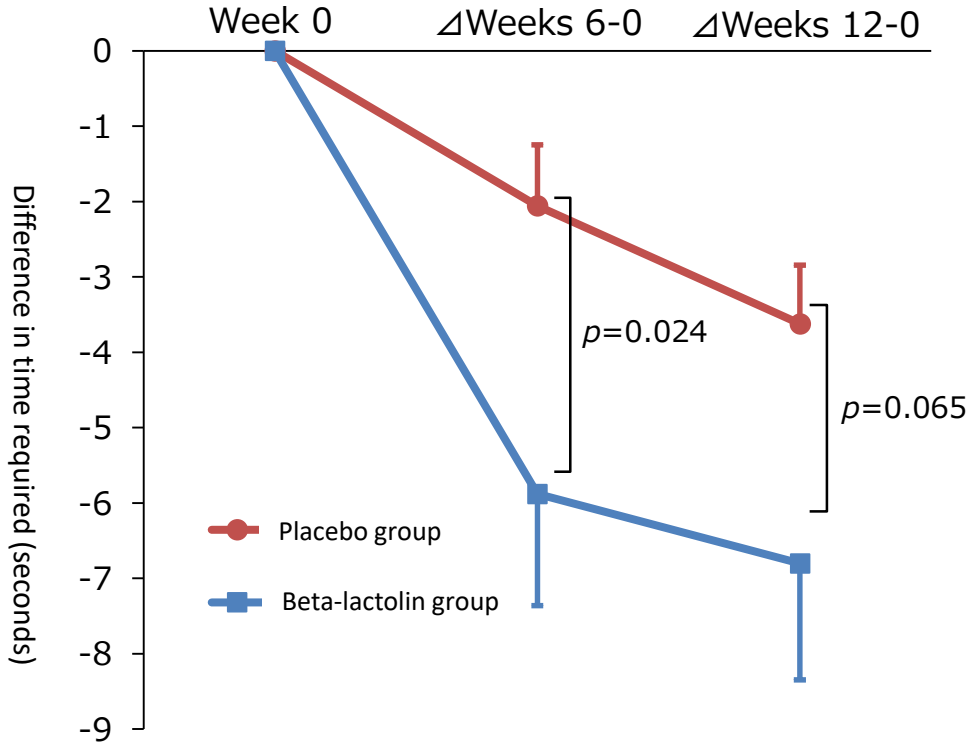




Bars represent means±SE, Placebo ; N=53, Beta-lactolin ; N=51

Improved performance in cancellation and detection test (executive function)

■Performance checked by having subjects identify specific characters from a variety of character strings



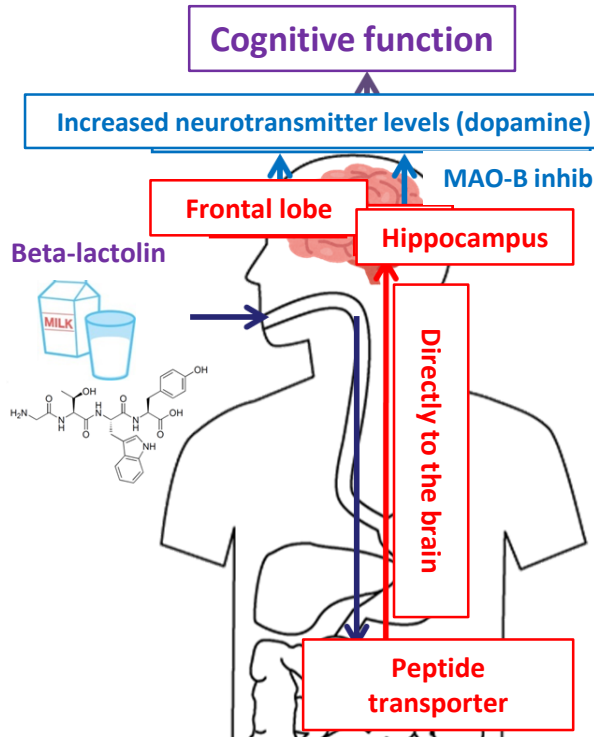
Kita et al., Front Neurosci, 2019

Uniqueness of this technique

Beta-lactolin exerts a positive effect on both memory and attention by reaching the brain and increasing neurotransmitter levels

■Comparison with other ingredients

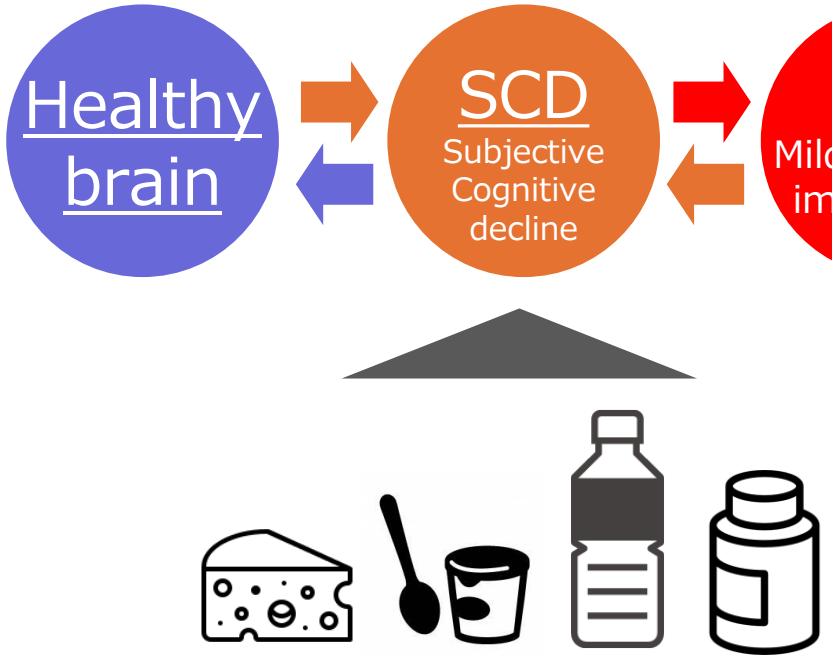
	DHA	Ginkgo biloba	Beta-lactolin	Commentary
Ingredients involved	DHA	Flavonoids Terpene lactones	GTWY(1.8mg)	Since a small amount of beta-lactolin can produce a large effect, its use may be expanded to a wide range of food and beverages
Background research	Mediterranean cuisine	None	Epidemiology and Camembert	New functions discovered as a result of epidemiological research
Effectiveness (in humans)	Memory improvement, Limitation of neutral fat	Memory improvement	Memory improvement Attention improvement Increased cerebral blood flow	Target ranges that can stimulate brain function are several
Mechanism of action	Hypermobility of cell membrane	Increased cerebral blood flow	Increased dopamine	Increases neurotransmitter dopamine, which directly regulates the improvement of cognitive function
Non-clinical evidence	Antioxidant, Anti-inflammatory Alzheimer's disease prevention	Antioxidant	Prevention of Alzheimer's disease, Anti-aging, Improvement of depression	May also potentially improve brain function



Reaches the brain and exerts its effect

Future potential

Achieve sustainable brain health support by combining nutrition with other solutions



Taking steps early on in the course of daily life will help preserve brain health

【Knowledge of dementia】
Education

【Visualization of brain health】
Identification (early action)

【Visualization of brain health】
Realization effect (motivation)

【Cognitive training】
Brain training, Education

【Connection with society/others】

【Exercise】

【Daily diet】
A balanced diet
Food and drink/supplements

KIRIN

FANCL KYOWA

Kyowa Hakko Bio's fermentation technology

What is fermentation technology?

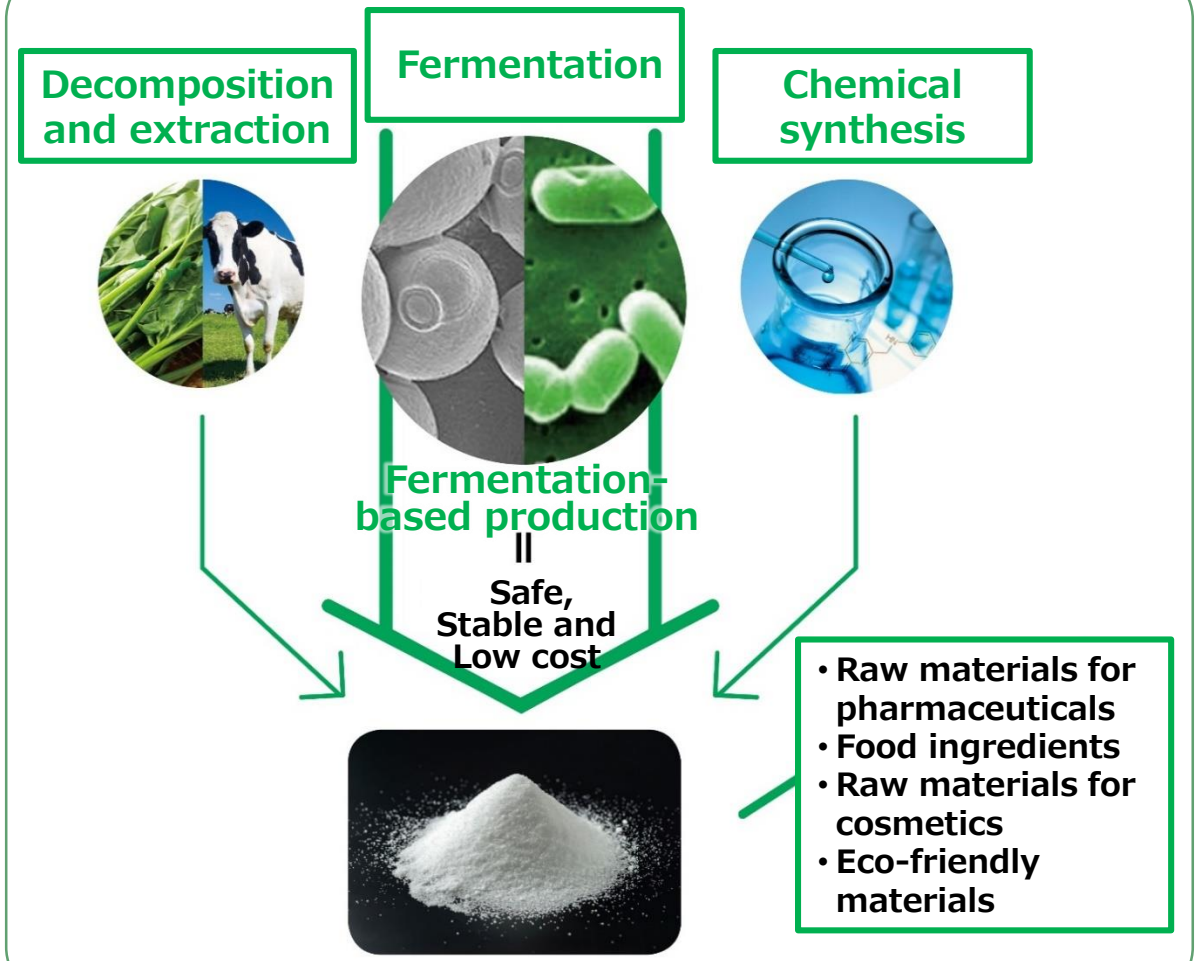
- Consists of having microorganisms produce amino acids and other valuable compounds, and then extracting these at a high level of purity

Advantages of fermentation technology

- Fermentation technology enables the safe, stable and low-cost (mass) production of valuable compounds.

- Chemical synthesis involves the use of hazardous substances and carries risks to the environment
- Extraction from plant and animal sources may undermine sustainable food supply amidst warnings of food crises due to global population growth

Advantages of fermentation

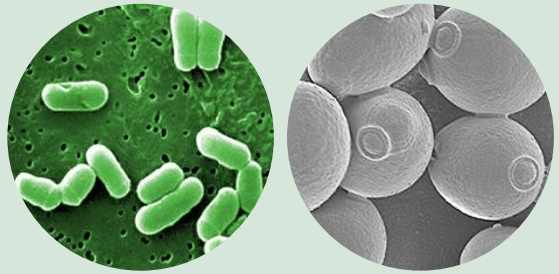


Kyowa Hakko Bio's core technologies

All three technologies (Microbial breeding technology, industrialization technology and environmental technology) are necessary for fermentation-based production.
Kyowa Hakko Bio has been refining and accumulating these technologies over the years.

Microbial breeding technology

Technology to create microorganisms that produce the desired ingredients



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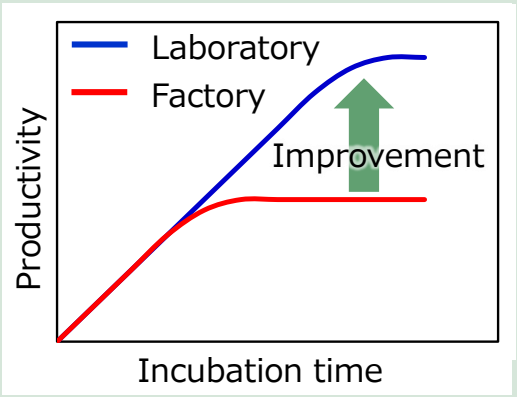
Industrialization technology

Technology to realize safe, secure, and large-scale production



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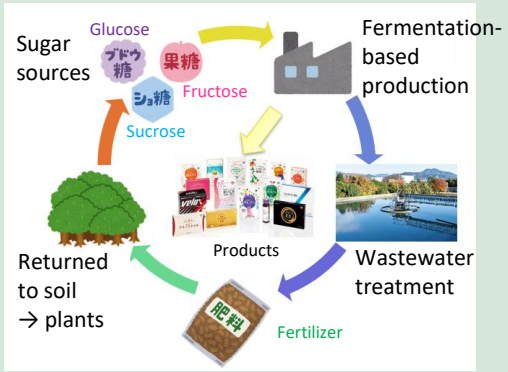
Environmental technology

Technology for reducing environmental impact and processing liquid waste



Microbial breeding technology

Technical issue: creating microorganisms capable of producing compounds on an industrial scale is difficult

Technological capabilities for solving this issue: we possess know-how on microbial breeding that enables establishing production systems and can be used for a variety compounds

Design

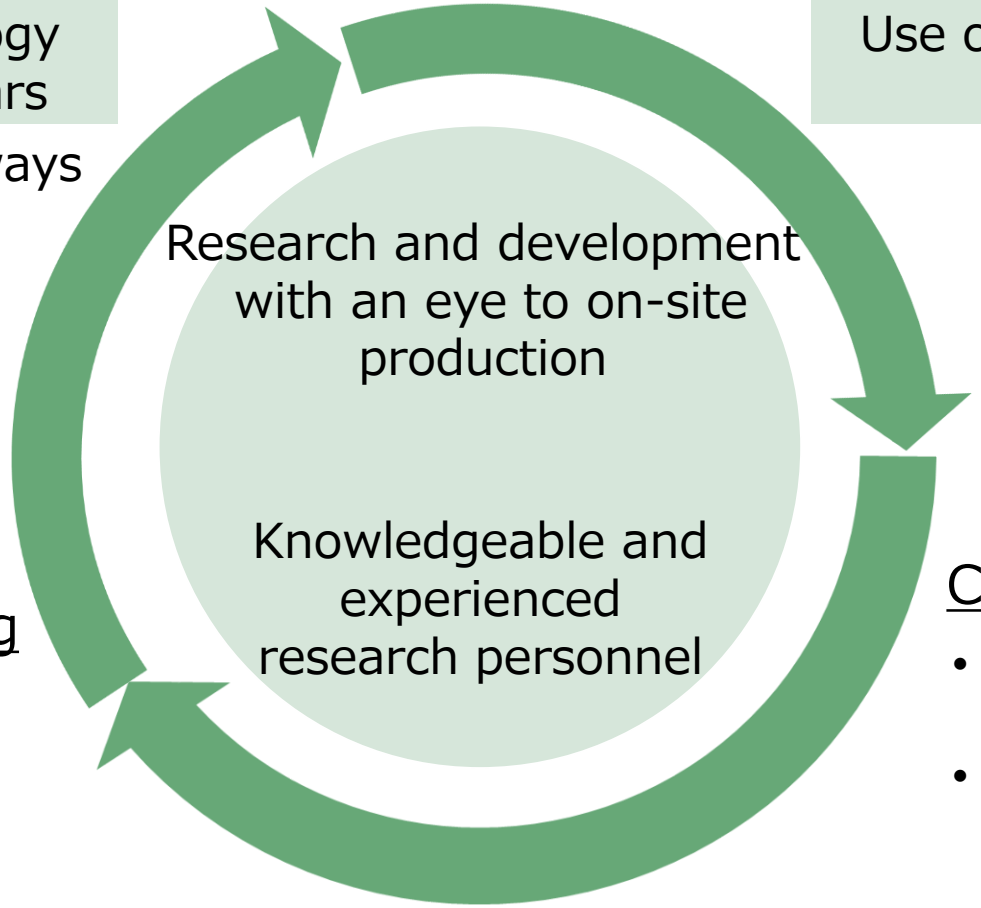
Metabolic control technology developed over many years

- Design of metabolic pathways for microorganisms

Hypothesis formulation
Improvement plan

On-site installation testing

- Cause analysis and analysis of gap between small- and large-volume cultures



Breeding

Use of findings from previous studies

- Genetic design
- Enzyme modification
- Transgenesis

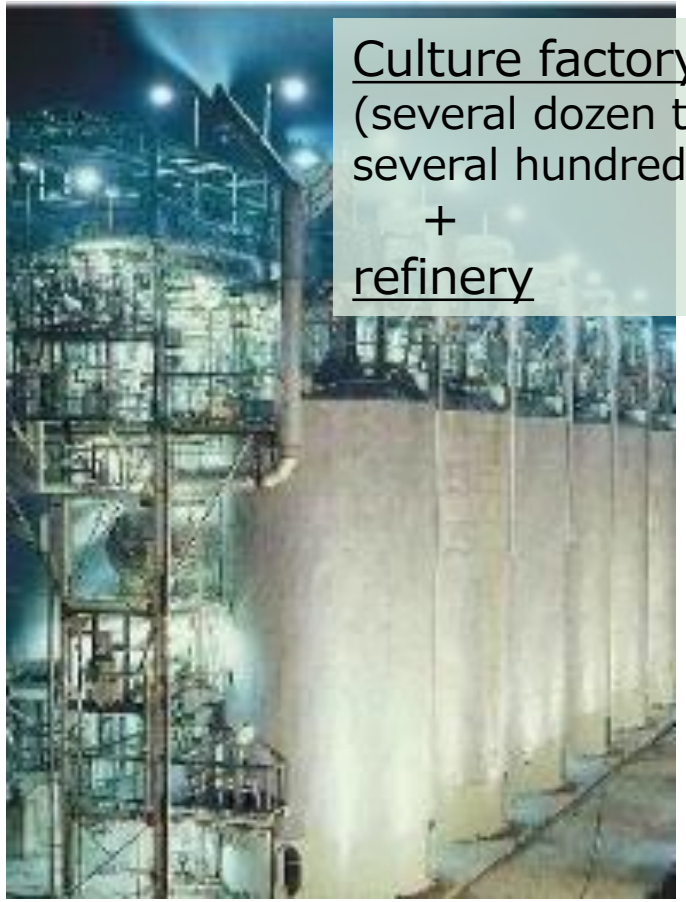
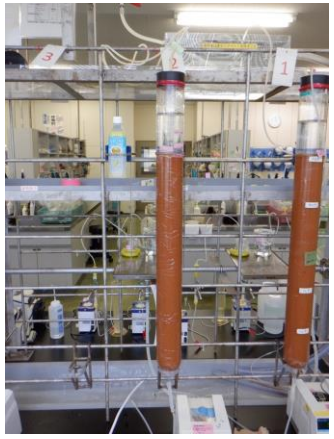
Culture and analysis

- Optimization of microbial culture
- Measurement of products and byproducts

Industrialization technology

Technical issue: stable production becomes more difficult as cultures grow in size

Technological capabilities for solving this issue:industrial-scale manufacturing achieved through technical verification with pilot facilities

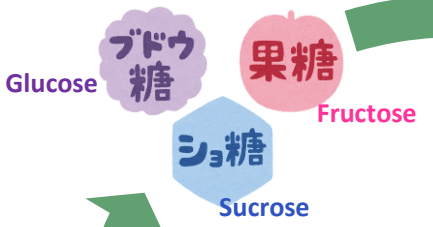
	Research laboratory	Pilot	Factory
Culture process	 Fermentor (capacity: several L)	 Culture facilities (several kL)	 Culture factory (several dozen to several hundred kL) + refinery
Refining process	 Purification equipment (laboratory)	 Purification facilities (pilot)	

Environmental technology

Technical issue: industrial production is not possible without efficient treatment technology for the waste liquids from fermentation

Technological capabilities for solving this issue: develop a highly efficient treatment process for industrial wastewater from fermentation in order to reduce the environmental impact

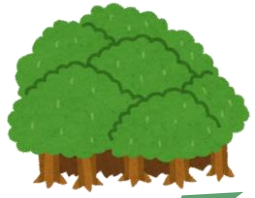
Sugar sources



Fermentation-based production

Products

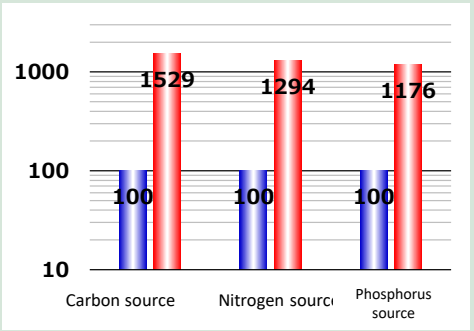
Wasterwater treatment



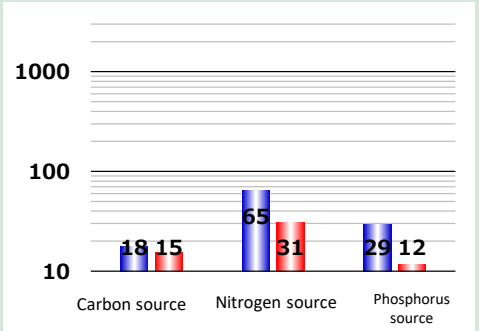
Returned to soil → Plants

Natural environment

Before wastewater treatment



After wastewater treatment



General domestic wastewater

Industrial wastewater from fermentation

Strict environmental standards for wastewater*

*These values are based on the 2016 Bureau of Waterworks Tokyo Metropolitan Government Report. The data are expressed in relative values, with carbon source, nitrogen source, phosphorus source in the domestic drainage of Tokyo being 100.

A history of creating and accumulating technologies

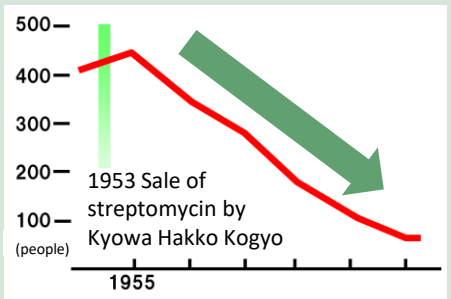
We have been pioneers in creating new technologies, aiming to use fermentation technology to solve the social issues

1951 *1956* *1958* *1993* *1998* *2000* *2004*

Helped eradicate tuberculosis

- Introduced technology for the production of streptomycin, a tuberculosis drug, to Japan in order to help reduce the number of tuberculosis cases

Number of tuberculosis cases per 100,000 people in Japan

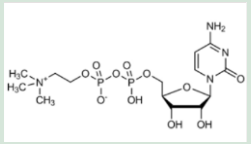


Developed the world's first fermentation-based technology for the production of amino acids

- **L-Glutamine**
  Revolutionized the seasoning industry
- **L-Lysine**
  A breakthrough in the history of livestock feed

➡ First corporate recipient of the **Japan Academy Prize**

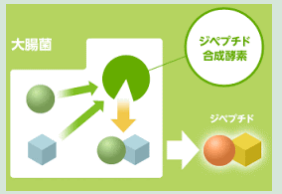
Developed technology for the bio-production of citicoline, a drug for the treatment of impaired consciousness



First in the world to develop oligosaccharide production technology

Developed dipeptide fermentation technology and established a production system

- Contributing to nutritional support for the sick through use in infusions



World's first successful production of human milk oligosaccharides by microorganisms

- Mass production technology to provide infants with powdered milk that is similar to breast milk, and adults with the health benefits of breast milk

Kyowa Hakko Bio's research and development system

Basic research (R&I Center)

- Develops new production processes using microbial breeding technology

Research on industrialization (Technical Research Laboratories)

- Uses microbial breeding technology, industrialization technology and environmental technology to create robust processes that enable actual production

Basic R&D data

- R&D expenses: 2.4 billion yen (in 2019)
- Researchers: 126 (as of April 2020)
- Number of patents (production process/crystallization): 119

▲ R&I Center* (Tsukuba City, Ibaraki Prefecture)
*Integrated into Kirin Holding's Kirin Central Research Institute, effective July 1, 2020.

▲ Technical Research Laboratories (Hofu City, Yamaguchi Prefecture)

Fermentation technology, the source of our competitive advantage, contributes to a sustainable society

As a result of continuously channeling R&D resources into the development of new materials using amino acid fermentation technology rather than the expansion of amino acid production, we have been able to introduce technically challenging, high value-added materials to the market.

Amino acid fermentation ⇢ Scaling up is not a strategy we pursue.

Core competencies

Technology for breeding microorganisms
- Creation of industrially viable microorganisms

Technology for establishing production systems
- Safe, secure and low-cost manufacturing processes

Environmental technology
- Technologies for reducing environmental impact

Focus on developing new materials

Strategy
- Technology development strategy aiming to contribute to society based on the concept of SDGs

R&D organization and researchers
- R&D organization founded upon fermentation technology
- A group of experts in biotechnology

Development of high value-added functional materials and entry into new areas of biotechnology

Research on citicoline, human milk oligosaccharides(HMO), dipeptides and gut bacteria

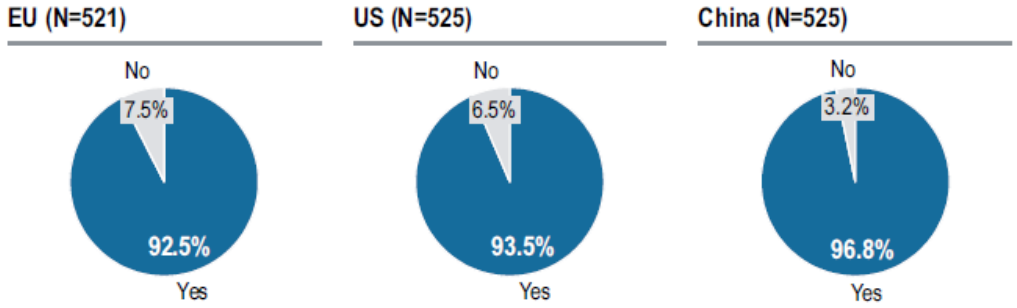
What can be expected from this technology

* Abbreviation of “Human Milk Oligosaccharides”

Manufacturing and supplying human milk oligosaccharides (HMO*) to contribute to the health and well-being of people around the world

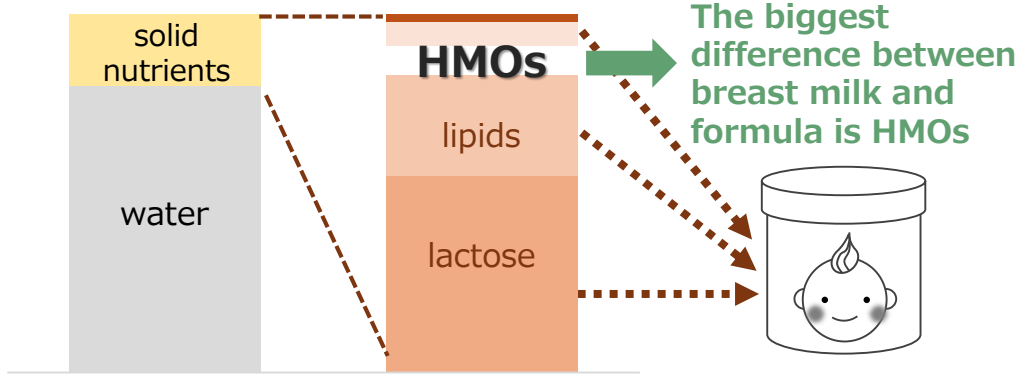
There is a need for milk powder that is similar to breast milk

Q. Do you want a formula that is similar to breast milk*?



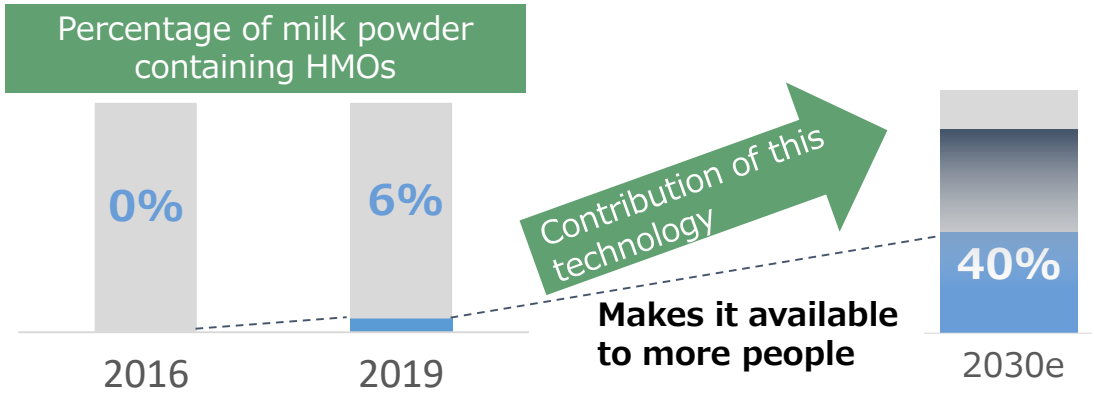
More than 90% of respondents want formula that is similar to breast milk

Nutrients in breast milk

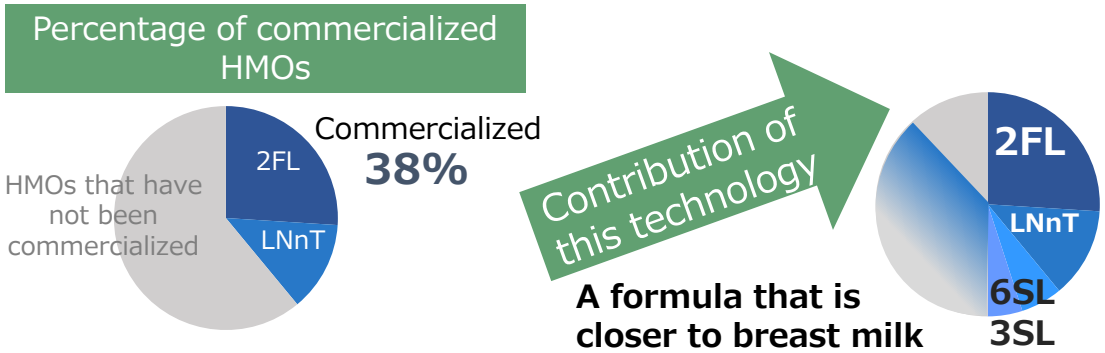


Greater variety of HMOs for larger population

■The percentage of formulas containing HMOs is still low.*



■ We still haven't covered HMOs in breast milk.**



* In-house data,
** Percentage accounted for by commercialized HMOs, 2FL and LNnT, out of breast milk HMOs

What can be expected from this technology

* Abbreviation of “Human Milk Oligosaccharides”

Manufacturing and supplying human milk oligosaccharides (HMO*) to contribute to the health and well-being of people around the world

HMOs found to be valuable for the health of adults as well

Recent years have seen the publication of an increasing number of functional studies on HMOs In particular, the effects of 6SL & 3SL on brain function is attracting attention.

* WHO Web site

Example of social issues to be solved: dementia

Dementia: Approximately 50 million people worldwide suffer from dementia.
Ten million people develop dementia every year.
One of the major causes of disability and dependency among older people worldwide*.

Technology overview 1: using innovative biotechnology to establish a process for the mass production of HMO

Challenges of conventional technology

Existing production method:
Chemical synthesis
- Expensive
- Complex process

What Kyowa Hakko Bio has made possible

Innovative production method:
Fermentation
- Low-cost
- Simple process

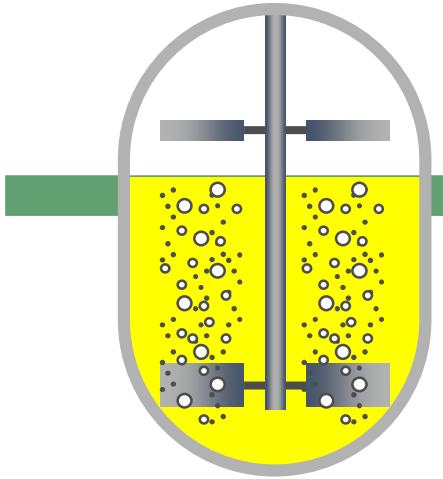
= enables large-scale production

Simple ingredients

- Sugars
- Materials for culture media

HMO-producing bacteria

Simple production method



Mass production

Technology overview 2: three types of HMO processes established thus far (2FL, 6SL, 3SL)

Strain Development

Developing Production Process

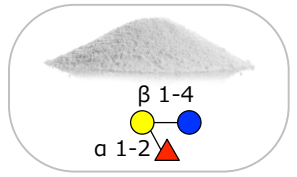
High content in breast milk

Technological breakthrough

2’-fucosyllactose (2FL)-producing bacteria

Fermentation and purification process appropriate for 2FL

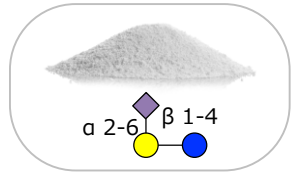
2FL product



6’-sialyllactose (6SL)-producing bacteria

Fermentation and purification process appropriate for 6SL

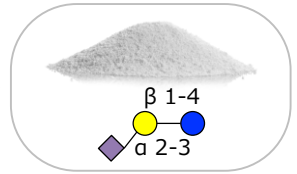
6SL product



3’-sialyllactose (3SL)-producing bacteria

Fermentation and purification process appropriate for 3SL

3SL product



Background of this technology (1): Kyowa Hakko Bio's strain development

2000 World's first microorganism-based HMO production process developed

Technology for inducing **high-yield** production by microorganisms

Metabolic engineering

1956 Developed the world's first amino acid fermentation method

1990 Developed biological production process for nucleic acids

Technology for inducing the production of **new substances** by microorganisms

Genetic engineering

2003 *C. glutamicum** genome determined

2004 Dipeptide synthase discovered

* A type of amino acid-producing bacteria

Applications filed for more than 14 HMO process patents

PATENT PROTECTED

Technologies accumulated by Kyowa Hakko Bio

Background of this technology (2): production process development by Kyowa Hakko Bio

Developing a process suitable for the production of raw materials for products to be consumed by infants, which require high quality similar to pharma-grade products

Fermentation process

The manufacturing process precisely controls the culture parameters of bacteria, which are sensitive to minute changes in raw materials and temperature, and controls impurities less than 0.1%

Purification process

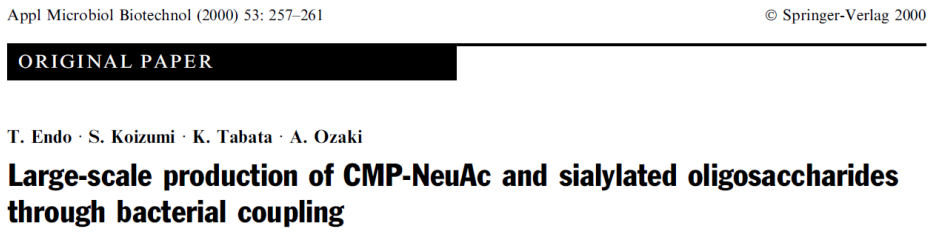
Our highly controlled refining process leverages our experience in pharmaceutical manufacturing, and is intended to ensure a steady supply of high-purity products

Uniqueness of this technology

Established the **world's first*** industrial-level HMO production system

Kyowa Hakko Bio is the first company in the world established an industrial-level production system for HMOs*

More than 140 citations since 2000**



Appl Microbiol Biotechnol (2000) 53: 257–261 © Springer-Verlag 2000

ORIGINAL PAPER

T. Endo · S. Koizumi · K. Tabata · A. Ozaki

Large-scale production of CMP-NeuAc and sialylated oligosaccharides through bacterial coupling

We have been pursuing a competitive edge by applying for production process patents

2FL production process: employs one proprietary patented technology

6SL production process: employs six proprietary patented technologies

3SL production process: employs six proprietary patented technologies

PATENT PROTECTED

* Tetsuo Endo et. al.,Appl. Microbiol. Biotechnol. 53, 257-261 (2000), https://link.springer.com/article/10.1007/s002530050017,
** Google Scholar

6SL and 3SL are not yet supplied at industrial level

Future possibilities

Bringing more varieties of HMOs and their health value to the world

The presence of as many as 250 different HMOs has been reported in breast milk
Only two types, however, are commercially available in the world today

We are researching production methods for more types of HMO
than just the ones whose commercialization has been scheduled (2FL, 6SL and 3SL)

Bringing formula that resembles breast milk more closely to babies around the world

Bringing the health value of breast milk to adults through health foods and beverages

What can be expected from this technology

Contribute to the brain health of people around the world in the fields of food & beverages to pharmaceuticals

Citicoline is a compound found in the body

Citicoline is needed for the maintenance of cell membranes in the brain and neurons

Cell membrane damage and quality deterioration as a result of trauma to the brain and aging

Decline in brain function

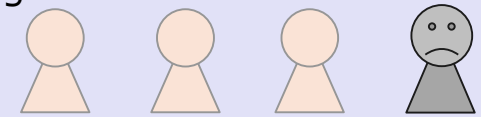
Maintenance of brain function

Kyowa Hakko Bio's citicoline

■Global market needs and main applications

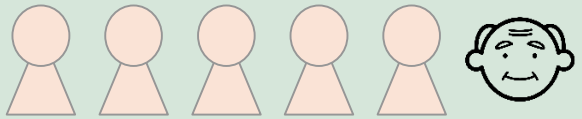
Number of stroke victims: 13.7 million*

Chances of having a stroke: one in four people over the age of 25.**



An ingredient for active pharmaceutical ingredients

65 years and older in Europe and the US: 18%,*** about one in six.



An ingredient for health foods

*World Stroke Organization "Learn about stroke"
** N Engl J Med. 379(25):2429-2437 (2018)
*** World Population Prospects 2019

What can be expected from this technology

Contribute to the brain health of people around the world in the fields of food & beverages to pharmaceuticals

Pharmaceutical use as treatments for brain disease

Health foods to **maintain** cognitive decline

Use in **food and beverages** to support brain power and skills

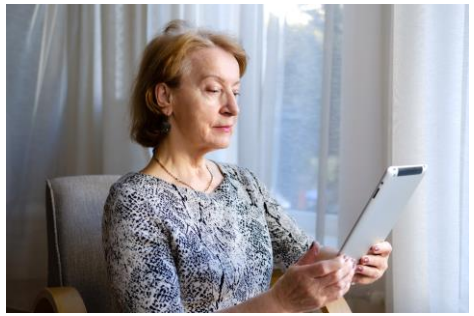
■ For the patients

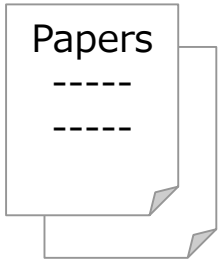
- Use as a treatment for stroke*
- Other benefits
  - Brain dysfunction from accidental trauma**
  - Glaucoma treatment ***
- A wide range of dosage forms, including injections, oral formulations and eye drops

*Curr Opin Investig Drugs 2(12), 1757-1762 (2001)
**From the Drug Interview Form
***Nutrients 12(3), 793 (2020)

■ For the middle-aged and elderly

- Support for improving cognitive function as it declines with age
- Studies on the effectiveness of citicoline for the elderly has been published in academic journals****





****Methods Find Exp Clin Pharmacol 19(3), 201-210 (1997)

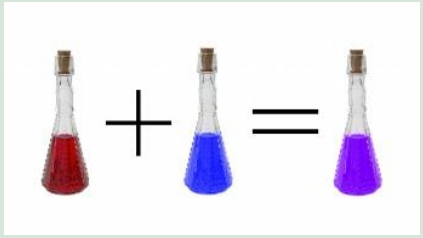
■ For young and mature customers

- Helping those who want to improve their work performance and efficiency
- Can be widely applied to convenient beverages and processed foods

Technology overview 1: established an innovative method for the mass production of citicoline based on an unique technology

Challenges of past technologies

The main technique used was chemical synthesis

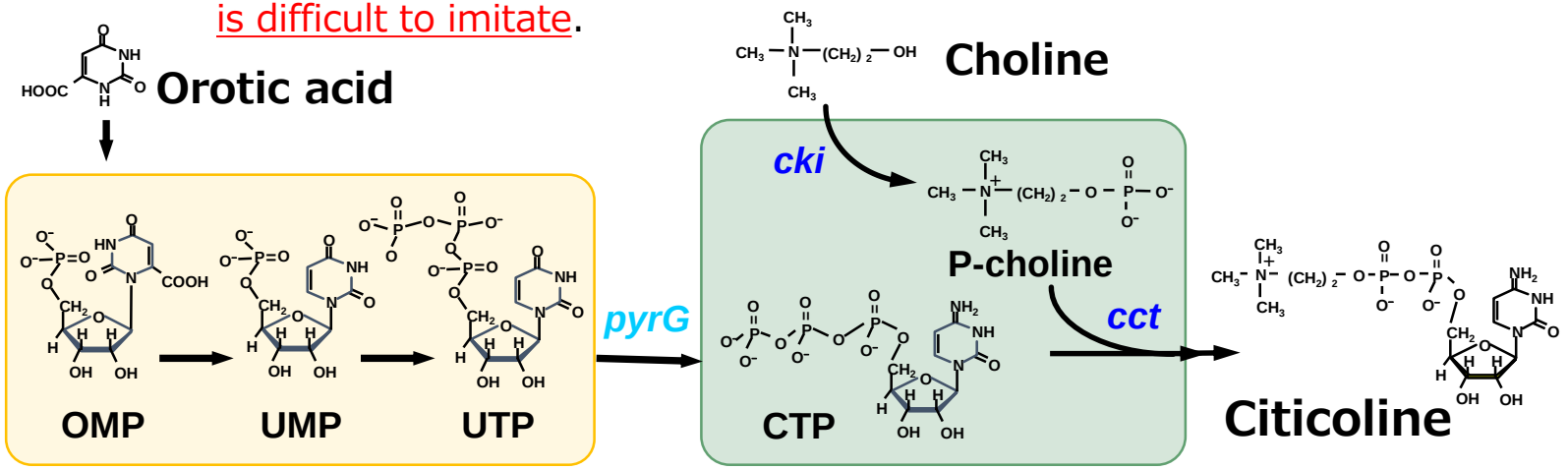


Expensive,
low-volume production

Difficult to ensure a steady, low-cost supply of citicoline for wide population

What Kyowa Hakko Bio has made possible

- We have designed an original process combining the nucleic acid fermentation technology developed over many years and microorganisms with enhanced enzymatic activity
- We have established a process that enables high quality product manufacturing in a plant scale
- Kyowa Hakko Bio, with its extensive knowledge of microorganisms, is uniquely able to provide citicoline inexpensively and in large quantities by using a production method based on its established know-how that is difficult to imitate.



Technology overview 2: a biological process combining conventional and new technologies

Nucleic acid fermentation technology

allowing simultaneous control of two microorganisms

Nucleic acid-producing bacteria

Production process developed by leveraging manufacturing technologies for active pharmaceutical ingredients

Inexpensive raw materials

Orotonic acid

Choline chloride

A simple process

Fermentation and purification process suitable for citicoline

Citicoline products

Citicoline biosynthesis-enhancing bacteria

Microbial design technology

Kyowa Hakko Bio is the only company that can provide a stable supply of citicoline with this technology

The History of Kyowa Hakko Bio and citicoline

1956 Kyowa Hakko developed the world's first microorganism-based amino acid fermentation technology

1970s **Citicoline produced via chemical synthesis** began to be distributed on the world’s markets as a pharmaceutical product

1990 **Kyowa Hakko Bio began producing citicoline through fermentation**

2003 Kyowa Hakko Bio started to actively promote citicoline under brand name Cognizin

Cognizin®
For the evolution of your mind®

2016 Cognizin has been introduced to a major, well-known health food brand in the US.

2016-2020 Demand for energy drinks is on the rise
Citicoline's concept - improved attention and concentration - meets market needs

Future We are planning to expand areas where Cognizin can be used as a food ingredient

The uniqueness of this technology

Provide added value in the form of citicoline produced by Kyowa Hakko Bio

Creation	Production	Sales	Use

■ Technical Research Laboratories

- Evaluation for improvements to manufacturing process, etc.

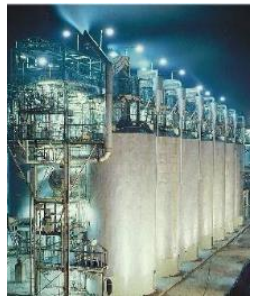
■ R&I Center

- Conduction of fundamental to clinical research in cooperation with research institute

Papers

- Manufacturing high quality pharmaceutical grade products in an appropriate facility environment

Safe & secure



Health benefits backed by science

■ As an active pharmaceutical ingredient

- Supplying to China, Europe, India and Southeast Asia

■ As a food/health food*

- Branded citicoline

Cognizin®

- Raising awareness on social media and websites (Twitter, Instagram, Facebook, etc.) * Mainly in the U.S. and Europe

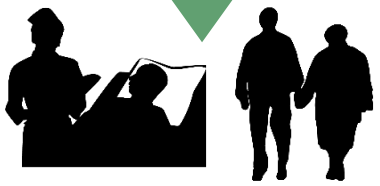
■ Hospitals

Treatment of patients

■ Foods and health foods

Effective dose of citicoline based on clinical studies

Health and happiness



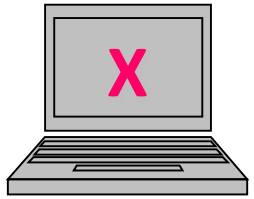
Investigate competitor, customer and market trends to reconsider productivity and obtain new data.

Uniqueness of this technology

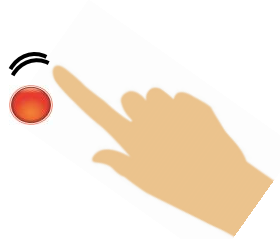
Effectiveness and application of citicoline scientifically proven by Kyowa Hakko Bio

Examples of Effects of citicoline intake

■ Clinical trials in healthy individuals



- Decreased omission error when operating a computer*



- Improved finger tapping test [1]**

[1] Number of times a button is tapped

■ Combined with other well-known ingredients

- Combination with DHA

➡ Further cognitive improvement can be expected***

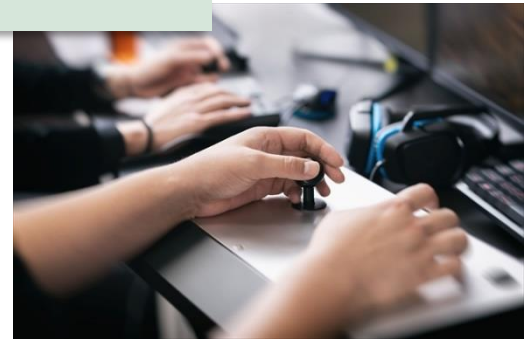
Proposals for new markets and areas

■ Providing Citicoline to people seeking better focus across generation

- Improve the work productivity of remote workers in their new lifestyle and maintain their focus

For the evolution of your mind !

- E-sports market, where finger tapping tasks and focus are important



* Food and Nutrition Sciences, 3: 769-773 (2012)
**J Atten Disord, 23(2), 121-134, (2019)
*** J Pharmacol Sci 139(4), 319-324 (2019) (non-clinical development)

Future vision

Contribute to support the brain health of more people

Kyowa Hakko Bio provides citicoline as branded ingredient for brain health backed by scientific evidence

Expanding the number of countries and regions where citicoline can be used as an ingredient for foods and health foods via ingredient registration procedures

High-quality, safe citicoline for “pharmaceutical”, and proven Cognizin brand citicoline for “foods and health foods”