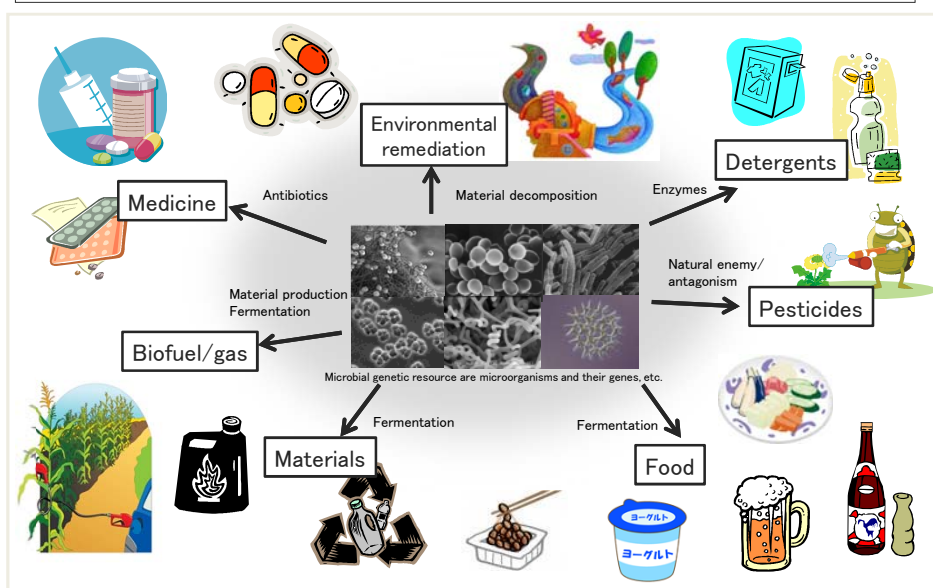


II. Microbial genetic resources

Category of use and application Type of microbial genetic resources	Safety/security	Innovation/ advanced technology	Industrial competitiveness/ core technology of manufacturing/ international expansion	Environment/ energy	Regulatory compliance
Mold	60, 61, 65 66, 69	60, 61 65, 66,	62-67	68	
Yeast	70		70-72		
Actinomycete	73, 74	73, 74	75		
Algae			76	77	
Bacteria	78, 85-91		78-84, 92	82, 85	86-89
Others	93, 94, 98		95-97		98, 99

What are microbial genetic resources?

Our lives are supported by helpful activities of microorganisms.



Microbial Genetic Resources

1. What are microbial genetic resources?

Microbial genetic resources are microorganisms and their genes. Microorganism is a general term for living organisms so small that their structures can only be observed through microscopes. It includes molds, yeasts, bacteria, algae, etc. It is estimated that only about 5% of approximately 3 million species of microorganisms said to exist on earth is actually known to us.

Utilization of microbial genetic resources is expanded to various industries such as medicine, chemistry, agriculture, food, and environment. In Japan, people have long been using advanced fermentation technologies that use microorganisms for making sake, miso, soy sauce, etc., and these technologies from traditional fermentation industry, such as breeding methods, have now been applied to broader industries such as medicine, chemistry, food, and environment.

For example, a microorganism that produces glutamic acid (an amino acid), a constituent of umami, was discovered by a Japanese, and the technology to produce amino acids using microorganisms has spread all over the world. Amino acids produced with this technology is now used for various purposes such as food, medicine, dietary supplement and cosmetics.

For another example, by using a microorganism which produces an enzyme called cellulase, synthetic detergents realizing dramatically improved detergency with much less amount of detergent and low environmental burden were produced. In addition to the use in detergents, enzymes from microorganisms are widely used in food processing as well.

2. Collecting microbial genetic resources in Japan

Microbial genetic resources are stored and used as own research materials in many universities, research institutes and enterprises. On the other hand, resource centers specialized in providing microbial genetic resources to third parties are, in most countries, managed by public organizations. In Japan, there are organizations such as the National Institute of Technology and Evaluation (NITE).

NITE preserves approximately 80,000 microorganisms and is one of the world's top-class microbial resource centers. The preserved microorganisms are used for many purposes such as product tests based on JIS, quality control of medicine based on

Pharmacopoeia, exploration of microorganisms producing useful compounds such as antibiotics and enzymes, and as references for evaluating pathogenicity, comparing productivity of materials, etc.

3. Demands for microbial genetic resources for a new era

For Japan to continue sustainable growth by overcoming various restrictions such as environment, energy and resources, it is necessary to realize industrial processes where the environmental burden is minimized by suppressing consumption of natural resources and recycling materials resulting from production and consumption processes.

Microorganisms and their enzymes can realize material production and material conversion with less energy which are difficult with chemical reactions; therefore, demands for various manufacturing processes and environmental measures using microorganisms are sure to increase, which contributes to sustainable society.

For fulfilling these demands, it is indispensable for the country to continuously collect microbial genetic resources as the infrastructure for research and development, for technological innovation and for manufacturing industry.

Furthermore, use of microbial genetic resources in the fields which are in line with national strategies, such as Green Innovation and Life Innovation, is required for the future. For example, existing biofuels made from plant materials such as corn, rape seed and palm have a problem of conflict with food. Microalgae, on the other hand, do not conflict with food, and are recently gaining attention as materials for biofuel, driving development competition throughout the world.

Under this circumstance, NITE, possessing approximately 80,000 microorganisms, actively works to expand microbial genetic resources based on users' demands, enrich functional information of microorganisms useful in industries, and provide information in easy-to-understand and easy-to-use manner, in order to support research and development of business operators on manufacturing processes and environmental measures using microorganisms, and thus to support promotion of industrial use of microorganisms.

II. Microbial genetic resources

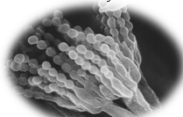
60. "Blue mold (medicine, etc.)"
 - Production of the world's first antibiotic from blue mold -
61. "*Monascus* (pharmaceutical material)"
 - Contributes to developing hypercholesterolemia medicine -
62. "*Monascus* (food)"
 - Production of food additives/supplements by *Monascus* -
63. "Koji mold (cosmetics)"
 - Mass production of skin-whitening kojic acid by Koji mold -
64. "Koji mold (host for material production)"
 - Development of mass-producing technology of xylanase -
65. "Koji mold (food)"
 - Production of traditional food and medicine by Koji mold -
66. "Koji mold (deferriferrichrysin)"
 - Mass production of deferriferrichrysin -
67. "*Trichoderma* (texture improvement of textile products)"
 - Used to improve texture of textile products -
68. "Parasitic mold (microbial pesticide)"
 - Used in microbial pesticide with low environmental burden and high level of safety -
69. "Mushroom (detecting poisonous mushroom)"
 - Developing a detection kit for poisonous mushrooms based on genome information -
70. "Yeast (biotechnology-based medicine)"
 - Manufacturing biotechnology-based medicine using fission yeast -
71. "Yeast (coenzyme Q10)"
 - Production of coenzyme Q10 by yeast -
72. "Yeast (bread)"
 - Breadmaking using yeast -
73. "Actinomycete (tacrolimus)"
 - Using immunosuppressive agents derived from actinomycete -
74. "Actinomycete (antibiotics)"
 - Production of antibiotics by actinomycete -
75. "Actinomycete (sweeteners)"
 - Manufacturing glucose isomerase using genetically modified actinomycete -
76. "Microalgae (functional food)"
 - Production of functional food using microalgae -
77. "Microalgae (biofuel)"
 - Production of novel biofuel using microalgae -
78. "*Corynebacterium* (amino acids)"
 - Manufacturing amino acids using *Corynebacterium* -
79. "Lactic acid bacteria (antiallergic effect)"
 - Contributes to ease allergy symptoms -
80. "Lactic acid bacteria (functional food)"
 - Production of GABA by lactic acid bacteria -
81. "Acetic acid bacteria (cellulose)"
 - Production of high-quality cellulose using acetic acid bacteria -
82. "*Bacillus* (enzymes)"
 - Used to produce synthetic detergents with low environmental burden -
83. "Ice nucleation active bacteria (artificial snow making)"
 - Used for artificial snow making material adopted in the Winter Olympics -
84. "*Bacillus subtilis* (high performance host)"
 - Genome-based design of high performance host -
85. "*Bacillus subtilis* (microbial pesticide)"
 - Used for microbial pesticide with low environmental burden and high level of safety -
86. "Test strain (evaluation of antibacterial properties)"
 - Contributes to quality assurance of antibacterial products -
87. "Test strain (evaluation of microorganism contamination)"
 - Contributes to safety assurance tests for medicine and medical equipment -
88. "*Mycoplasma* (quality assurance of products)"
 - Contributes to quality assurance of reagents etc. -
89. "*Escherichia coli* (raw meat treatment)"
 - Contributes to safe supply of raw meat -
90. "*Salmonella* (rapid identification)"
 - Developing quick identification kit for *Salmonella* using genome information -
91. "Genome information (*Staphylococcus aureus*)"
 - Enables early diagnosis of MRSA infection -
92. "Genome information (anaerobic hyperthermophilic archaea)"
 - Production of reagents for molecular biology research -
93. "Genome information (influenza virus (1))"
 - Predicting the annual epidemic strain of influenza -
94. "Genome information (influenza virus (2))"
 - Monitoring swine-origin novel influenza virus in 2009 -
95. "Genome information (aerobic hyperthermophilic archaea (1))"
 - Used in a research which was awarded the Nobel Prize -
96. "Genome information (aerobic hyperthermophilic archaea (2))"
 - Discovering new knowledge in basic life science -
97. "Genome information (aerobic hyperthermophilic archaea (3))"
 - Production of inositol-1-phosphate synthase -
98. "Genome information (*Pseudomonas putida*)"
 - Safety evaluation based on genome information -
99. "Genome information (classification of microorganisms)"
 - Simple and reliable molecular phylogenetic classification based on gene sequences -

60. Examples of utilizing blue mold (medicine, etc.)

Blue mold

Blue mold is a general term for molds which belong to the genus *Penicillium*. As in its name, its color is blue and it is one of commonly seen molds.

The world's first antibiotic (penicillin) was discovered from this mold.



Blue mold

(National Institute of Technology and Evaluation (NITE), NBRC)

Functions of blue mold

[Production of medicine]

Produces antibiotics and agents which lower blood cholesterol level.

[Fermentation]

Urges fermentation and maturation of cheese and adds special flavors and tastes.

Formation of growth inhibition circle by antibiotics



(National Institute of Technology and Evaluation (NITE), NBRC)

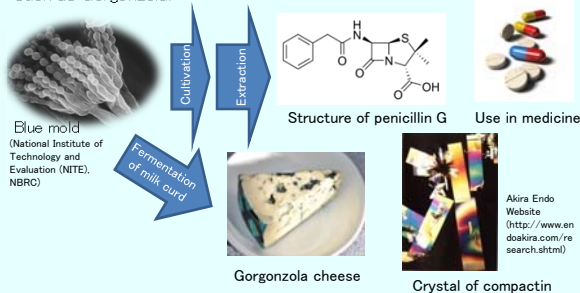
Production of the world's first antibiotic from blue mold

Penicillin produced by a blue mold is **the world's first antibiotic to be discovered**, and has saved many wounded soldiers from infectious diseases during World War II.

One of the greatest discoveries in the 20th century

From **compactin** which is produced by a blue mold, **the first statin (homologue of compactin) was developed**. Statins are administered to nearly 40 million patients everyday as a medicine specific for prevention and treatment of coronary artery disease and cerebral stroke, and are called "the world's most sold medicine".

As more familiar application, it is used in manufacturing cheese such as Gorgonzola.



Reference: Scientific American (<http://www.nikkei-science.com/?p=17930>)

61. Examples of utilizing *Monascus* (pharmaceutical material)

What is *Monascus*?

Genus *Monascus* includes several mold species.

It has long been used in Japan, China and Taiwan for producing Chinese rice wine and also as a coloring material. In recent years, bioactive compounds such as "monacolin K" produced by this mold have also been gaining attention.



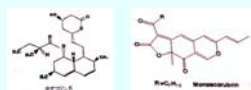
Akira Endo Website (<http://www.endoakira.com/research.shtml>)

Monascus pilosus NBRC4520, etc.

Developing useful functions of *Monascus*

[Production of useful compounds such as monacolin K]

Produces many useful compounds such as monacolin K which lowers the level of cholesterol, monascorubin which suppresses gastric cancer, and γ -aminobutyric acid which lowers blood pressure.



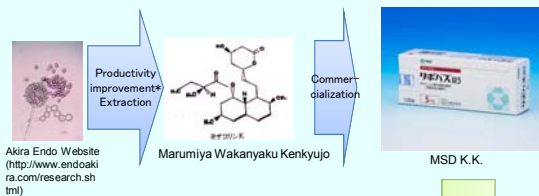
Marumiya Wakanyaku Kenkyujo

Contributes to developing hypercholesterolemia medicine

○ Pharmaceutical material

Monacolin K, found in *Monascus*, is reported to lower the level of cholesterol by inhibiting synthesis of cholesterol in the body.

Statin drugs used for hypercholesterolemia in recent years (Mevalotin (Sankyo), Lipovas (Banyu), etc.) were developed from monacolin K.



* There is a case where the productivity was improved by breeding a NBRC strain (patent application 2007-271481).

Lowering the level of cholesterol



References: Industrial Property Digital Library (http://www2.ipdl.inpit.go.jp/begin/BE_DETAIL_MAIN.cgi?Type=1&Menu=1&Bpos=1&Pos=6&Color=1&File=TimeDir_18/mainstr1337246609721.mst&Time=1337247655)
Interesting health information from Dr. Aun (<http://www.naoru.com/benikouji.htm>)

62. Examples of utilizing *Monascus* (food)

What is *Monascus*?

Genus *Monascus* includes several mold species.

It has long been used in Japan, China and Taiwan for producing Chinese rice wine and also as natural coloring. In recent years, bioactive compounds such as "monacolin K" produced by this mold have also been gaining attention.

Monascus pilosus NBRC4520, etc.



Akira Endo Website
(<http://www.endoakira.com/research.shtm>)

Developing useful functions of monascus

[Fermentation]

Because of its unique color, it has long been used as a microorganism for making fermented food.

[Production of color]

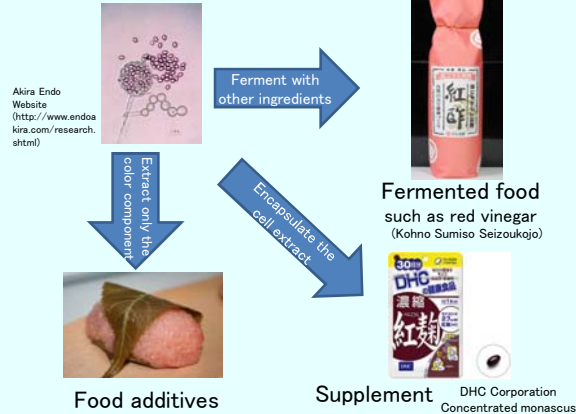
As in its name *beni koji* (red koji), it produces red color, which is used as natural coloring.



Akira Endo Website
(<http://www.endoakira.com/research.shtm>)

Production of food additives/supplements by *Monascus*

It is added to foods as an ingredient for red vinegar, Taiwanese fermented tofu and various other fermented foods, and as red food coloring (main components: ankaflavin and monascorubrin). Furthermore, it is commercialized as a dietary supplement in virtue of various confirmed health effects.



Reference: Interesting health information from Dr. Aun (<http://www.naoru.com/benikoji.htm>)

63. Examples of utilizing Koji mold (cosmetics)

What is Koji mold?

Mold species such as *Aspergillus oryzae*, which is widely used in traditional fermentation industries in Japan. It is used for brewing sake, making miso and soy sauce, etc.

In 2006, it was designated as a national fungus by the Brewing Society of Japan.

Furthermore, it is expected to be used in various fields of biotechnological industries such as use as the source of proteins including various enzymes or the host organism for producing recombinant proteins, and decomposition and recycling of biodegradable plastics and biomass.



(National Institute of Technology and Evaluation (NITE), NBRC)

Providing genome information of *Aspergillus oryzae*

- 12,074 genes consist the genome.
- *Aspergillus oryzae* (ki-koji) has intrinsic ability to actively secrete proteins such as hydrolytic enzymes. By comparing genome information with its two closely-related species, it was found that the number of genes related to intake of sugars and amino acids are specifically increased, confirming that *Aspergillus oryzae* is an ideal microorganism for fermentation.
- Predict genes related to kojic acid synthesis which has excellent skin-whitening effect.

Mass production of skin-whitening kojic acid by Koji mold

- Since the discovery of kojic acid (1900), it has attracted researchers with enthusiasm toward the white and smooth hands of craftsmen who handle koji.
- Though the whitening effect of kojic acid is excellent, increasing the expression level was considered indispensable to be able to compound it into cosmetics, so research and development were performed for its commercialization.

Researches for producing kojic acid

Genes essential to synthesize kojic acid in *Aspergillus oryzae* genome.

Genes for kojic acid synthesis

Identifying genes necessary to synthesize kojic acid from the genome and increasing their expression level realized its commercialization.

Multiplex genes for kojic acid synthesis

Genes for kojic acid synthesis

Genes for kojic acid synthesis

Genes for kojic acid synthesis

Commercialization

- Whitening cosmetics are commercialized by Kose Corporation.
<http://plaza.rakuten.co.jp/bio21/diary/201101120000/>
- Patent application 2010-64965, date of application: Mar. 19, 2010
- Patent application 2010-246532, date of publication: Nov. 4, 2010



Image picture

64. Examples of utilizing Koji mold (host for material production)

What is Koji mold?

Mold species such as *Aspergillus oryzae*, which is widely used in traditional fermentation industries in Japan. It is used for brewing sake, making miso and soy sauce, etc.

In 2006, it was designated as a national fungus by the Brewing Society of Japan.

Furthermore, it is expected to be used in various fields of biotechnological industries such as use as the source of proteins including various enzymes or the host organism for producing recombinant proteins, and decomposition and recycling of biodegradable plastics and biomass.



Providing genome information of *Aspergillus oryzae*

- 12,074 genes consist the genome.
- *Aspergillus oryzae* (ki-koji) has intrinsic ability to actively secrete proteins such as hydrolytic enzymes. By comparing genome information with its two closely-related species, it was found that the number of genes related to intake of sugars and amino acids are specifically increased, confirming that *Aspergillus oryzae* is an ideal microorganism for fermentation.
- This ability makes *Aspergillus oryzae* a suitable host for material production.

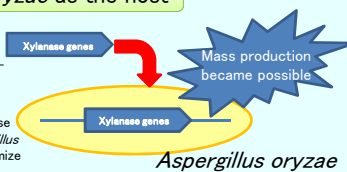
Development of mass-producing technology of xylanase

- "Xylooligosaccharide" obtained by decomposing polysaccharide derived from seaweed was reported to have anticancer bioactivity. *Nature* (Vol. 464, pp. 908-912, 2010)
- Using *Aspergillus oryzae* for mass-producing xylanase, xylooligosaccharide which induces the death of cancer cells was produced from seaweed. http://www.gekkeikan.co.jp/company/news/201009_01.html

Obstacle for use Production level of xylanase using procaryotes as the host was too low to fulfill industrial needs.

Using *Aspergillus oryzae* as the host

- Identify xylanase genes from the genome of hyper-thermophilic bacterium.
- Introduce xylanase genes to *Aspergillus oryzae* and optimize the expression.



Success in developing mass-producing technology of xylanase by using high protein secretion ability of Koji mold.

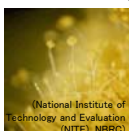
65. Examples of utilizing Koji mold (food)

What is Koji mold?

Mold species such as *Aspergillus oryzae*, which is widely used in traditional fermentation industries in Japan. It is used for brewing sake, making miso and soy sauce, etc.

In 2006, it was designated as a national fungus by the Brewing Society of Japan.

Furthermore, it is expected to be used in various fields of biotechnological industries such as use as the gene source or the host organism for producing recombinant proteins including various enzymes, and decomposition and recycling of biodegradable plastics and biomass.



Functions of *Aspergillus oryzae*

- 12,074 genes consist the genome.
- *Aspergillus oryzae* (ki-koji) has intrinsic ability to actively secrete proteins such as hydrolytic enzymes. By comparing genome information with its two closely-related species, it was found that the number of genes related to intake of sugars and amino acids are specifically increased, confirming that it is an ideal microorganism for fermentation.
- *Aspergillus oryzae* is a microorganism which produces and releases various enzymes from the tip of its hyphae, and has a strong nature to decompose starch to glucose and proteins to amino acids on steamed rice or steamed barley, which is its culture medium.

Production of traditional food and medicine by Koji mold

Koji, made by growing microorganisms such as *Aspergillus oryzae* on rice, rice bran, barley, wheat and soybeans, is used in the production of miso, soy sauce, sake, salted koji, etc.

Furthermore, diastase, an amylolytic enzyme produced by *Aspergillus oryzae*, is compounded in stomachic and digestive drugs.



66. Examples of utilizing Koji mold (deferriferrichrysin)

What is Koji mold?

Mold species such as *Aspergillus oryzae*, which is widely used in traditional fermentation industries in Japan. It is used for brewing sake, making miso and soy sauce, etc.

In 2006, it was designated as a national fungus by the Brewing Society of Japan.

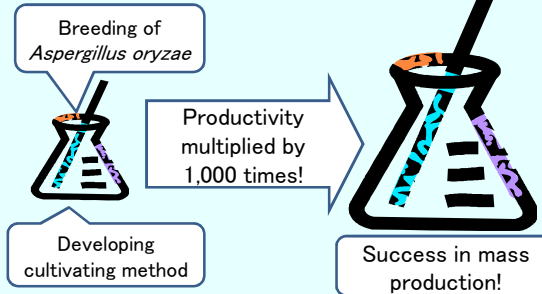
Furthermore, it is expected to be used in various fields of biotechnological industries such as use as the source of proteins including various enzymes or the host organism for producing recombinant proteins, and decomposition and recycling of biodegradable plastics and biomass.



Deferriferrichrysin

- *Aspergillus oryzae* produces "deferriferrichrysin".
- When deferriferrichrysin binds with iron in sake, it develops reddish orange color, so that it has been an unwanted chemical substance in the sake industry.
- However, deferriferrichrysin has various functions such as anti-inflammatory, antioxidation and skin-whitening effect.

Mass production of deferriferrichrysin



It is expected to serve for a wide variety of purposes such as a material for medicine, functional food and cosmetics.



Medicine



Functional food



Cosmetics

67. Examples of utilizing *Trichoderma* (texture improvement of textile products)

What is *Trichoderma*?

Genus *Trichoderma* includes several mold species which decompose woods and other cellulosic biomass. It is noted to have high cellulose decomposition activity (produces abundant cellulase).



Developing useful functions of *Trichoderma*

[Cellulose decomposing enzymes]

Cellulase refers to a group of enzymes which decompose cellulose, a major component of plant cell wall. It is produced by *Trichoderma* and many other microorganisms. It is used for many purposes such as the use as an ingredient of laundry detergent, for processing foods, and for processing

Used to improve texture of textile products



Extract enzymes

Commercialization

Cellulase liquid enzyme agent Enzyon S-KTL

(Rakuto Kasei Industrial Co., Ltd.)

Use

Functions of enzyme agent

- Loosens fiber to let fabric softener to easily permeate, and **softens the texture**.
- Fiber becomes **lighter**.
- Melts and **removes the nap** on the surface of cellulose materials such as cotton, hemp and rayon to improve the texture.



Biowash Texture improvement

* Polypeptide which enhances functions of cellulase (derived from microorganisms, patent application 2010-122388) is also being developed. It can be used as an additive agent when using cellulase.

68. Examples of utilizing parasitic mold (microbial pesticide)

What is *Verticillium lecanii*?

A species of mold in the genus *Verticillium*. It is a representative insect parasite infecting a wide range of hosts such as Lepidoptera, Coleoptera (earwigs, etc.) and Hemiptera.



Verticillium lecanii

Provided by Arysta LifeScience Corporation

Developing useful functions of mold

Once *Verticillium lecanii* is attached to the body surface of a plant pest such as whitefly, which causes damages to vegetables and flowers, it is known to pass through the epidermis of the pest to enter its body and grows using water and nutrient in the body.

This kills the pest and suppresses agricultural damages by whiteflies.

Moreover, *Verticillium lecanii* is confirmed to be noninfectious to natural enemy insects.

Used in microbial pesticide with low environmental burden and high level of safety

Whiteflies, thrips, aphids, etc. are known as pests which give feeding damages to vegetables and flowers, and they have been causing great losses in agricultural products.

To solve this problem, a microbial pesticide using the insecticidal property of *Verticillium lecanii*, a mold, was developed as a novel ecologically friendly pesticide.



Verticillium lecanii

Provided by Arysta LifeScience Corporation



(Arysta LifeScience Corporation)



Whitefly infected by *Verticillium lecanii*

Provided by Arysta LifeScience Corporation

Among microbial pesticides, 26 products to control pests which cause damages to agricultural products are being sold (as of Sept. 2008).

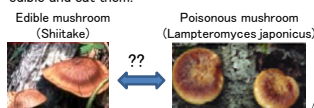
Compared to chemical pesticides, microbial pesticides which use microorganisms from nature have merits such as less impact on the environment, high level of safety and less frequent emergence of resistance in pests toward the pesticide, and they are expected to be used in environmentally-sustainable agriculture.

69. Example of utilizing mushroom (detecting poisonous mushroom)

What are poisonous mushrooms?

A great number of mushrooms (4,000 to 5,000 species) are said to exist in Japan, and edibility of the majority is unknown. In Japan, about 100 species are known to be edible, and about 40 are poisonous. Mushroom poisoning accounts for 70% of poisoning by natural toxins, and accounts for 60% of the cases which result in deaths.

This is because distinguishing poisonous mushrooms requires great skill, and ordinary people may misidentify poisonous mushrooms as edible and eat them.



Detection using DNA

Poisonous mushrooms are generally identified visually, however, their identification requires expertise and the form of the fruiting body (the cap of a mushroom) must be retained for confirmation.

So far, methods for specific detection of microorganisms such as bacteria and fungi using DNA are known, but none has been reported as a detection method for poisonous mushrooms such as *Lampteromyces japonicus*.

Developing a detection kit for poisonous mushrooms based on genome information

A detection method specific to poisonous mushrooms using genome information of mushrooms was developed.

- Quick detection in only 1.5 hours became possible.
- Detection from cooked mushrooms became possible.



Tottori University Research Profile Search 2012
"Effective use of unused mushrooms"

- Since identification of the species of eaten poisonous mushroom was difficult, only symptomatic treatment was possible for those who had mistakenly eaten them, but by using this method and identifying the mushroom, treatment best for the species of poisonous mushroom will become possible.

70. Example of utilizing yeast (biotechnology-based medicine)

What is fission yeast?

Fission yeast is a kind of yeast with its genetics well characterized. Since its properties such as the manner of fission are similar to those of higher organisms, it has been actively used for researches in the fields of molecular genetics and cell biology as a model for cell division.

Furthermore, its safety level is high, being used for brewing traditional alcoholic beverages such as wine, rum and kvass.



Fission yeast
Schizosaccharomyces pombe

Functions of fission yeast

The genome sequence of fission yeast was almost completely decoded in 2002 as the sixth eucaryote, and has been proven to have molecular genetic functions similar to those of higher animals.

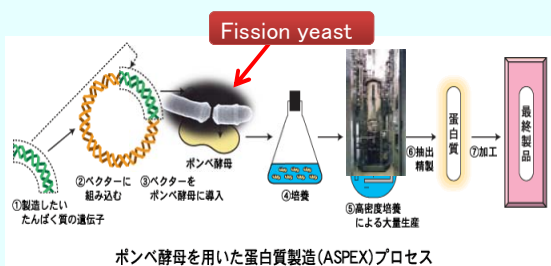
Therefore it has especially high potential for recombinant expression of genes derived from higher animals such as human.

Manufacturing biotechnology-based medicine using fission yeast

○ Biotechnology-based medicine

Biotechnology-based medicine is a medicine manufactured by using biotechnology such as DNA recombination and cell fusion. Some examples are insulin used as a diabetic medicine and interferon used for cancer and hepatitis C.

The market for biotechnology-based medicine has exceeded 75 billion dollars in 2007, and is likely to continue expanding along with the progress in drug development technologies and the increase of aging population. On the other hand, manufacturing biotechnology-based medicine usually requires higher cost, and demands for highly effective production technology of high quality recombinant proteins have been rising, with fission yeast being one solution.



Reference: Ministry of Economy, Trade and Industry (<http://www.meti.go.jp/committee/summary/0004631/report02.pdf>)

71. Examples of utilizing yeast (coenzyme Q10)

What is yeast?

Yeast is a general term for microorganisms that are eucaryotic and unicellular. It multiplies by budding or fission. It performs alcohol fermentation through glucose metabolism.

As it is used in bread-making and brewing alcoholic beverages (beer, wine, etc.), it contributes greatly to the dietary life of humans.

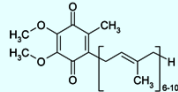
Recently it is also used to produce coenzyme Q10.



Fission yeast
Schizosaccharomyces pombe

Coenzyme Q10

Ubiquinone (coenzyme Q10), one of the electron carriers found in the membranes of mitochondria and prokaryotic cells, has antioxidant effect, but its production in the body reaches the peak in the 20's and declines with age.



Structure of ubiquinone
(coenzyme Q10 has 10 isoprene units)

Production of coenzyme Q10 by yeast

Coenzyme Q10, a molecule indispensable in energy production of living cells, has widely been used in functional health food and cosmetics as the most important antioxidant.

There are two ways to manufacture coenzyme Q10: by chemically processing coenzymes extracted from plants, and by fermenting with microorganisms such as yeast. The latter allows to produce only the natural form (*cis* form) of coenzyme Q10. Japan accounts for almost 100% of the share for production of coenzyme Q10 in the world.



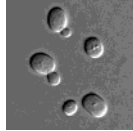
Reference: Japanese Coenzyme Q Association (<http://www.coenzymeq-jp.com/index.html>)

72. Examples of utilizing yeast (bread)

What is yeast?

Yeast is a general term for microorganisms that are eukaryotic and unicellular. It multiplies by budding or fission. It performs alcohol fermentation through glucose metabolism.

As it is used in bread-making and brewing alcohols (beer, wine, etc.), it contributes greatly to the dietary life of humans.



Functions of yeast

Yeast for breadmaking is generally called the baker's yeast. Through alcohol fermentation, it raises the dough by decomposing sugar into ethanol and carbon dioxide. The ethanol evaporates from the dough as it is heated.

* In 2000 BC in Mesopotamia, bread was already being made using yeast.



Breadmaking using yeast

Even within the same species of microorganism, there are different characteristics, and accordingly, different final products can be made. The following example is on yeast which suppresses the growth of contaminating bacteria, but there are yeasts with other useful characteristics such as richer flavor and quicker fermentation.



Culture collection of own company, NBRC, etc.

Compare



Screening for yeast with wanted characteristics: Searching for yeast with the ability to suppress the growth of contaminating bacteria.

Commercialization
Marketing



Use

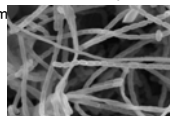


Suppressing other bacteria allows longer time to be spent for processing, enabling wider variety of bread to be made.

73. Examples of utilizing actinomycete (tacrolimus)

What is *Streptomyces tsukubaensis*?

Streptomyces tsukubaensis, isolated from the soil of Mount Tsukuba in Tsukuba, Ibaraki, is an actinomycete belonging to the genus *Streptomyces*, and produces tacrolimus (FK506) which suppresses the activity of the immune system.

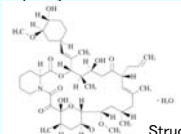


Provided by
Astellas Pharma Inc.

Streptomyces tsukubaensis

Immunosuppressive substance tacrolimus

It is a macrolide compound with strong immunosuppressive effect which was discovered in the culture medium of *Streptomyces tsukubaensis*.



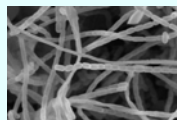
Structure of tacrolimus

Provided by Astellas Pharma Inc.

Using immunosuppressive agents derived from actinomycete

Tacrolimus, an immunosuppressive substance, suppresses rejection which is a problem in patients with an organ or marrow being transplanted.

It was approved as a suppressor of rejection for liver transplantation in 1993, and is currently used as an immunosuppressive drug indispensable after transplantation of marrow or organ such as liver, kidney and heart. Furthermore, it is used for treating rheumatoid arthritis, atopic dermatitis and other autoimmune diseases.



Streptomyces tsukubaensis
Provided by Astellas Pharma Inc.

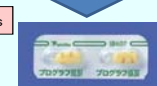
Cultivate

Purify
tacrolimus



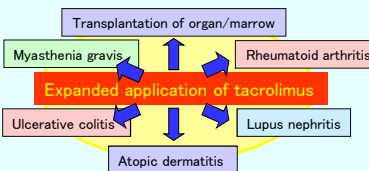
Crystal of tacrolimus
Provided by Astellas Pharma Inc.

Commercialization



Astellas guide for drugs
(<http://www.astellas.com/jp/health/product/photo/prg/prg.05.html>)

Immunosuppressive drug Prograf
(active ingredient: tacrolimus)

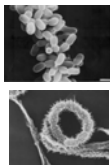


* Sold in 97 countries as of June 2011.

74. Examples of utilizing actinomycete (antibiotics)

What is actinomycete?

An actinomycete is a mold-like bacterium with radially stretching filamentous hyphae. It is widely distributed in soil and other natural environment. Some are pathogenic, but there also are useful ones such as *Streptomyces* which produce various antibiotics (streptomycin, etc.).



(National Institute of Technology and Evaluation (NITE), NBRC)

Functions of actinomycete

Actinomycetes, especially those belonging to the genus *Streptomyces*, produce various antibiotics (metabolites of microorganisms such as streptomycin and chloramphenicol, which suppress the activity of other living organisms).

Moreover, many anticancer agents, enzymes, immunosuppressive agents, etc. derived from actinomycetes have been commercialized.

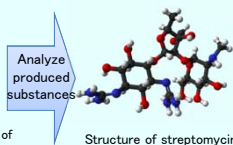
Anticancer agent: Mitomycin (Kyowa Hakko Kirin Co., Ltd.)



Production of antibiotics by actinomycete

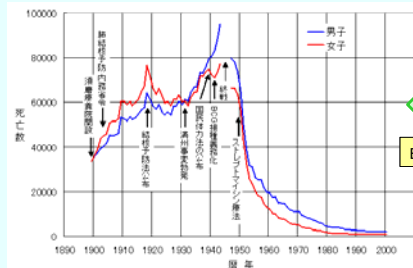


Isolation and cultivation of various actinomycetes



Streptomycin (Meiji Seika Pharma Co., Ltd.)

Transition in the number of annual deaths due to tuberculosis



Provided by Tokyo Metropolitan Institute of Public Health

The number of deaths from tuberculosis, once called the national disease in Japan and was the cause of over 100,000 annual deaths, has decreased dramatically after the introduction of treatment using streptomycin which was discovered from the culture medium of *Streptomyces griseus*.

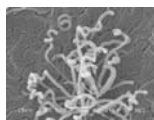
Establish treatment

75. Examples of utilizing actinomycete (sweeteners)

What is actinomycete?

An actinomycete is a mold-like bacterium with radially stretching filamentous hyphae. It is widely distributed in soil and other natural environment. Some are pathogenic, but there also are useful ones such as *Streptomyces* which produce various antibiotics (streptomycin, etc.).

Streptomyces rubiginosus, a species of actinomycete, produces an enzyme called glucose isomerase.



Actinomycete belonging to *Streptomyces*

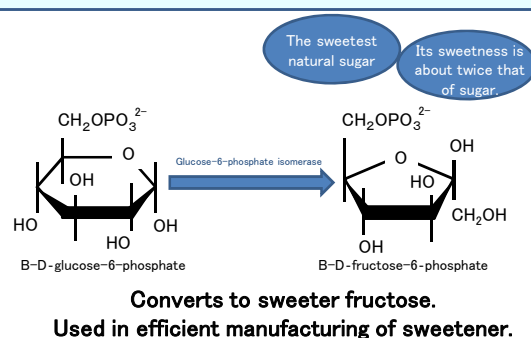
[Picture provided by] National Institute of Technology and Evaluation (NITE), NBRC

Glucose isomerase

- An enzyme which catalyzes the conversion of glucose into fructose (fruit sugar).
- Since fructose has strong sweetness and its taste is favored by many people, it is industrially synthesized using this enzyme.

Manufacturing glucose isomerase using genetically modified actinomycete

Immobilized glucose isomerase is produced by genetic modification of *Streptomyces rubiginosus* (Product name: GENSWEET). It is sold as an enzyme by Genencor.



76. Examples of utilizing microalgae (functional food)

What are microalgae?

Microalgae are phytoplankton with chlorophylls which assimilate carbon dioxide in the air and produce oxygen through photosynthesis. They lie at the bottom of food chains and provide the basis of the whole food web on earth.

Moreover, they are recently attracting attention as micro-organisms which contribute to reduce carbon dioxide, a greenhouse gas.



Volvox

(National Institute of Technology and Evaluation (NITE), NBRC)

Developing useful functions of microalgae

Some microalgae are rich in nutrients essential for humans to live such as vitamins, minerals, amino acids, carotenoids and unsaturated fatty acids. Therefore, they are used as ingredients for functional foods, animal feeds, etc.

Production of functional food using microalgae

○ Euglena Co., Ltd.

Euglena Co., Ltd. is a venture company with advanced cultivation technology of microalgae, centering on *Euglena*.

The company is expanding the technology to various fields such as functional foods, cosmetics, animal and aquaculture feeds and jet fuels, utilizing useful characteristics of *Euglena* such as high protein content, high nutritional value and high productivity of hydrocarbons.

[Company information]

Establishment: 2005

Capital: 460,650,000 yen

Main stockholders: Itochu Corporation, JX Nippon Oil & Energy Corporation, All Nippon Airways Co., Ltd., etc.)

Recent accomplishments: Received "METI Minister Award" in "JAPAN Venture Award 2012", etc.



Euglena

Functional food



Reference: Euglena Co., Ltd. (<http://www.euglena.jp/>)

77. Examples of utilizing microalgae (biofuel)

What are microalgae?

Microalgae are phytoplankton with chlorophylls which assimilate carbon dioxide in the air and produce oxygen through photosynthesis. They lie at the bottom of food chains and provide the basis of the whole food web on earth.

Moreover, they are recently attracting attention as micro-organisms which contribute to reduce carbon dioxide, a greenhouse gas.



Volvox

(National Institute of Technology and Evaluation (NITE), NBRC)

Developing useful functions of microalgae

Microalgae are known to produce many hydrocarbons, fatty acids and polysaccharides. Since various fuels can be produced from these substances, they have been gaining attention of the industry as a novel biofuel.

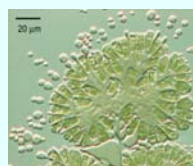
Production of novel biofuel using microalgae

In contrast to existing biofuels made from corn, rape seed, palm, etc. which have a problem of competing with food, microalgae, having higher oil productivity than those land plants with a possibility to relieve competition with food, are recently gaining attention as alternative materials for biofuel.

Currently, this field is highly competitive throughout the world, and accelerating research and development is considered important.

[Main microalgal fuel development projects involving Japanese enterprises]

Microalgae subjected to R&D	Enterprise	University
<i>Fistulifera</i>	Electric Power Development Co., Ltd., Yamaha Motor Co., Ltd.	Tokyo University of Agriculture and Technology
<i>Pseudochoirycystis ellipsoidea</i>	Denso Corporation, Toyota Motor Corporation, MicroAlgae Corporation, etc.	Chuo Univ., Kyoto Univ., Ochanomizu Univ., Saga Univ.
<i>Pseudochoirycystis ellipsoidea</i>	Denso Corporation	Chuo Univ.
<i>Euglena gracilis</i>	JX Nippon Oil & Energy Corporation, Euglena Co., Ltd.	Keio Univ.
<i>Botryococcus braunii</i>	JFE Engineering Corporation	Univ. of Tsukuba
<i>Botryococcus braunii</i>	IHI Corporation, G> Y.K., Neo-Morgan Laboratory Incorporated	



Botryococcus braunii
M.M. Watanabe Lab., University of Tsukuba

The photo is an image.



Biofuel

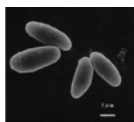
Reference: Council on Competitiveness-Nippon "Development of fuels using microalgae" Final report (<http://www.cocn.jp/>)

78. Examples of utilizing *Corynebacterium* (amino acids)

What is *Corynebacterium*?

A genus of club-shaped bacteria. They are distributed in various environments, and some species are indigenous to human body such as on the respiratory tract mucosa.

There are species which produce exotoxin like *Corynebacterium diphtheriae* and are pathogenic to animals or humans, but some are known to produce large amounts of amino acids.



Corynebacterium glutamicum

Useful functions of *Corynebacterium glutamicum*

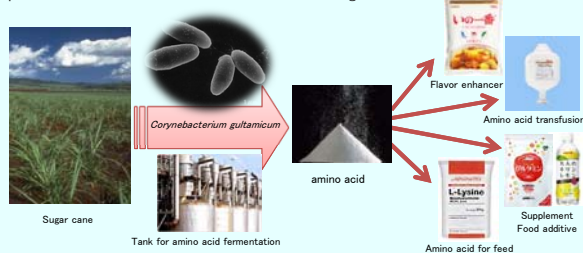
Corynebacterium glutamicum, a species of the genus *Corynebacterium*, was found by a Japanese company as a microorganism which produces large amounts of amino acids.

For over 50 years, it has been used for industrial production of amino acids for food, feed and medicine, and is the foundation of amino acid industry.

Manufacturing amino acids using *Corynebacterium* – Amino acid fermentation technology developed in Japan –

Twenty kinds of amino acids are known to be the constituents of proteins. Various biological and chemical functions have been discovered for them, and they are used for many industrial purposes such as flavor enhancers, medicines, food supplements, feed additives, chemicals and cosmetics.

The method to produce amino acids using microorganisms (fermentation method) was developed in Japan, a country with a traditionally advanced fermentation technology. The amino acid fermentation industry is expanding globally, and the production volume of amino acids is in an increasing trend.



The market for amino acids was approximately 1.3 million tons in 1996, reached approximately 2.6 million tons in 2005, and is still growing by an annual increase rate of few percent. The boom in healthy food etc. also had a significant influence, and the size of the market is growing even today.

Reference: Systematic Survey of the Technical Development of Fermentative Production of Amino acids (Center of the History of Japanese Industrial Technology, National Museum of Nature and Science) (<http://its.hakaku.go.jp/diversity/document/system/pdf/042.pdf>)
Ajinomoto Co., Inc.: Pictures of *Corynebacterium*, amino acid for feed, amino acid Ajinomoto Pharmaceuticals Co., Ltd. HP: Picture of amino acid transfusion
Kyowa Hakko Bio Co., Ltd. HP: Pictures of supplement and food additive Kirin Kyowa Foods Co., Ltd. HP: Picture of flavor enhancer

79. Examples of utilizing lactic acid bacteria (antiallergic effect)

What are lactic acid bacteria?

Lactic acid bacteria refer to microorganisms which produce lactic acid in general. They contribute to fermenting food such as yogurt and some tea products.

Some lactic acid bacteria are indigenous to digestive organs such as intestines, and serve to maintain homeostasis, protecting the body from other pathogenic microorganisms.



Bioplan Co., Ltd.
(<http://bioplan.co.jp/bi/seibutsu/microbiology.html>)

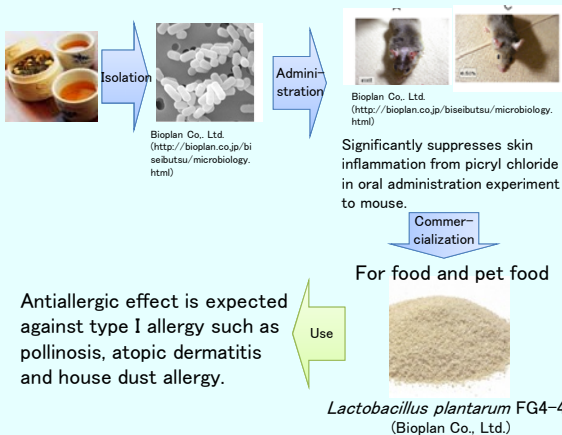
Developing useful functions of lactic acid bacteria

[Allergy suppression]

Some lactic acid bacteria are known to remove or suppress immune cells. Moreover, lactic acid bacteria are known to have a great influence on the intestinal environment, and even taking them through food is effective for the suppression.

Contributes to ease allergy symptoms

A species of enteric lactic acid bacteria suppresses immune cells which cause allergy symptoms.



* Patent pending (patent application 2010-133552)

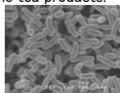
Reference: Industrial Property Digital Library (http://www.2ipdl.ipdl.go.jp/begin/BE_DETAIL_MAIN.cgi?Type=0&Menu=1&Bpos=1&Ppos=1&File=TimeDir_15/mainstr1337323427273.mst&Time=0)

80. Examples of utilizing lactic acid bacteria (functional food)

What are lactic acid bacteria?

Lactic acid bacteria refer to microorganisms which produce lactic acid in general. They contribute to fermenting food such as yogurt and some tea products.

Some lactic acid bacteria are indigenous to digestive organs such as intestines, and serve to maintain homeostatis, protecting the body from other pathogenic microorganisms.



(Kitasato University Research Organization for Infection Control Sciences HP)

Developing useful functions of lactic acid bacteria

Some lactic acid bacteria produce GABA*.

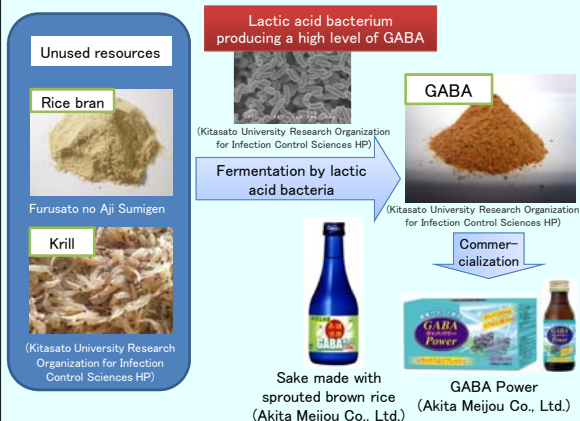
GABA is an amino acid found in the central nervous system of humans which serves as a neurotransmitter. It is known to have various bioactivities such as anti-stress and relaxing effect, suppressing blood pressure increase, anti-obesity effect, activating kidney and liver functions, and promoting alcohol metabolism.

* The formal name for GABA is γ -aminobutyric acid (gamma-aminobutyric acid)

Production of GABA by lactic acid bacteria

GABA produced from lactic acid bacteria is used in a variety of food products including dietary supplements, nutritional drinks, alcoholic and non-alcoholic beverages and chocolates.

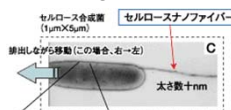
The current production methods of GABA include a method employing unused resources such as rice bran, and it contributes to create a new industry which integrates different fields such as collaboration with the brewing industry.



81. Examples of utilizing acetic acid bacteria (cellulose)

What is *Acetobacter xylinum*?

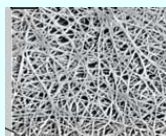
A species of acetic acid bacteria and is the most popular cellulose synthesizing bacterium. It synthesizes cellulose fiber (bacterial cellulose) through fermentation of sugars such as glucose.



Cited from Graduate School of Engineering, Hokkaido University PR brochure "Engineer Ring" No. 376

Developing useful functions of acetic acid bacteria

Cellulose synthesized by bacteria. The thickness of its fiber is 1/1000 of plant cellulose, and it has excellent physical properties such as high crystallinity, high elasticity and high absorability.

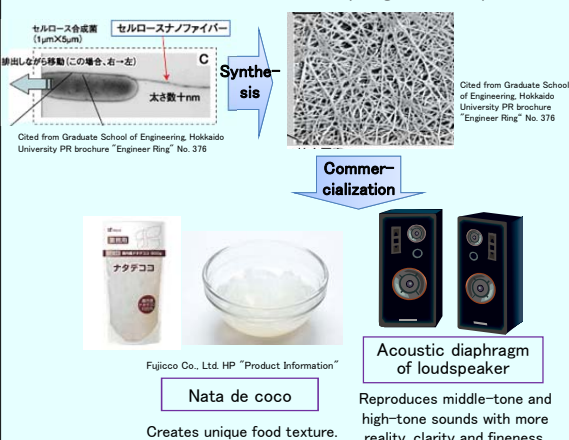


Cited from Graduate School of Engineering, Hokkaido University PR brochure "Engineer Ring" No. 376

Production of high-quality cellulose using acetic acid bacteria

Food and material

Nata de coco is made from bacterial cellulose. Due to its unique structure and physical property, bacterial cellulose is applied as various materials such as for acoustic diaphragms of loudspeakers.



References: A to Z Nanotechnology News, Articles, Directory and more (<http://www.azonano.com/news.aspx?newsID=8590>)
 Graduate School/Faculty of Engineering, Hokkaido University PR brochure "Engineer Ring" Issue of January 2009 ◆ Bio-nanofiber (<http://www.eng.hokudai.ac.jp/engineering/2009-01/feature0901-03.html>)
 Research on nanofiber control of bacteria cellulose (<http://www.noastec.jp-kinouindex-data2007-pdf-01-W07.pdf>)

82. Examples of utilizing *Bacillus* (enzymes)

What is *Bacillus*?

Bacillus is a major genus of bacteria living in the soil, air and water, which includes useful species such as *Bacillus subtilis* and *Bacillus subtilis* var *natto*. Furthermore, due to its nature to secrete various enzymes such as amylase, cellulase and protease, it is widely used in industrial production of enzymes.



Bacillus sp.
(National Institute of Technology and Evaluation (NITE), NBRC)

Development of useful functions of *Bacillus*

[Cellulose decomposing enzymes (cellulase)]

Cellulase refers to a group of enzymes which decomposes cellulose, a major component of plant cell wall.

When added to laundry detergents, it directly acts on the fiber molecules trapping dirt and stain, and melts a part of the fiber molecules to free the dirt and stain (a shift of cleaning paradigm from acting on the "dirt" to acting on the "fiber").

Used to produce synthetic detergents with low environmental burden

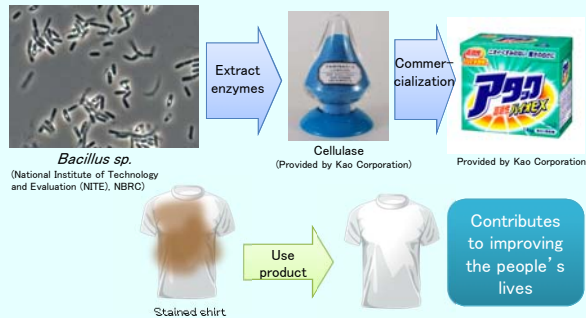
○ Effect of adding enzymes to detergent

Adding cellulase to synthetic detergents, the main products in the domestic market for laundry detergents, dramatically improved their cleaning performance even with smaller amount of the detergent.

This effect has been promoting products with low environmental burden such as decrease in contamination by rinse water due to reduction in the volume of detergent used, and use of smaller amount of resources in packing containers.

○ Enzyme market

The size of the global market for enzymes excluding medical and research use is 270 billion yen. Use in food processing (as saccharification agent for shochu and sake, as tenderizer for meat, etc.) accounts for the greatest portion, and use in detergents follows (in 2004).

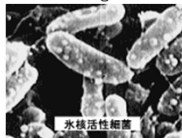


Reference: Global market of enzymes (<http://www.amano-enzyme.co.jp/jp/company/pdf/7/theme-6.pdf>)

83. Examples of utilizing ice nucleation active bacteria (artificial snow making)

What are ice nucleation active bacteria?

Ice nucleation active bacteria are microorganisms with ice nucleation activity which makes water to freeze easily. They cause frost damages and are harmful to plants, but recently they are actively researched for their application.



Provided by Laboratory of Microbial Technology, Department of Biotechnology, Faculty of Engineering, Kansai University

Developing useful functions of ice nucleation active bacteria

It produces ice nucleation active materials which consist of ice nucleating proteins, sugars, lipids and polyamine on the outer surface of the cells, and allows fine water droplets to freeze at a relatively high temperature of -2°C without supercooling.

Used for artificial snow making material adopted in the Winter Olympics

○ Artificial snow making material

In the Calgary Winter Olympics in 1988, sterilized cells of ice nucleation active bacteria were used as an artificial snow making material. It is also commercialized in Japan, and is used in ski parks.



Provided by Laboratory of Microbial Technology, Department of Biotechnology, Faculty of Engineering, Kansai University

Sterilization
Commercialization



Provided by Laboratory of Microbial Technology, Department of Biotechnology, Faculty of Engineering, Kansai University

○ Additive for frozen food

Using ice nucleation active bacteria to control the freezing speed prevents tissue destruction of frozen food.

Since sterilized cells of ice nucleation bacteria can be used, it is safe to be added to food.

* An ice nucleation active bacterium, *Xanthomonas campestris*, is approved by the Ministry of Health, Labour and Welfare as a food additive.

References: Laboratory of Microbial Technology, Department of Biotechnology, Faculty of Engineering, Kansai University (<http://www.bio.kansai-u.ac.jp/Microbial/hyoukaku.html>)
Patent Licensing Information Database/Utilization of patent information search (http://pldb.inpit.go.jp/pldb/html/HTML_R/2001/007/L2001007350.html)

84. Examples of utilizing *Bacillus subtilis* (high performance host)

What is *Bacillus subtilis*?

It lives in withered grass, soils, etc., being widespread in nature. Since it produces enzymes which decompose carbohydrates, it is widely used in the food industry. "Natto-kin" is classified in this species of bacteria.

For a microorganism used as the host for recombinant expression, improvement in material productivity and expansion of the variety of produced materials has been a challenge.

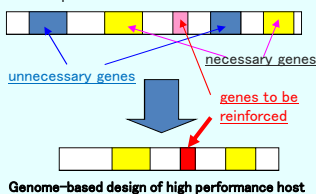


Reference: New Energy and Industrial Technology Development Organization (NEDO)

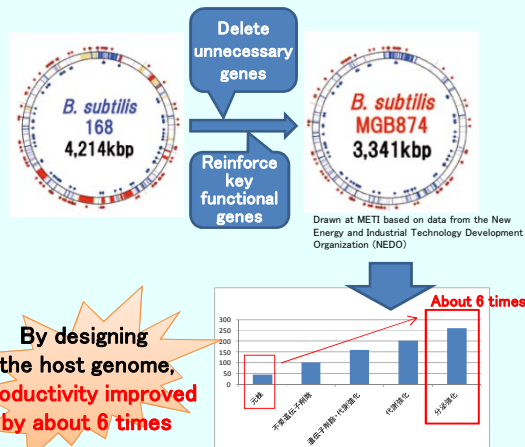
Providing genome information of *Bacillus subtilis*

Genome analysis allowed us to understand the host microorganism at the gene level.

It became possible to discriminate between necessary and unnecessary genes as a host for material production.



Genome-based design of high performance host



It is applicable not only for an effective production of natural compounds, but also for combinatorial biosynthesis of unnatural compounds.

* *Bacillus subtilis* Minimum Genome Factory (NEDO project)

85. Examples of utilizing *Bacillus subtilis* (microbial pesticide)

What is *Bacillus subtilis*?

It lives in withered grass, soils, etc., being widespread in nature. Since it produces enzymes which decompose carbohydrates, it is widely used in the food industry. "natto-kin" is classified in this species of bacteria.

For a microorganism used as the host for recombinant expression, improvement in material productivity and expansion of the variety of produced materials has been a challenge.



Bacillus subtilis
(National Institute of Technology and Evaluation (NITE), NBRC)

Developing useful functions of *Bacillus subtilis*

Bacillus subtilis has an extremely high proliferation potential. As the environmental condition worsens due to high temperature or low availability of nutrients, it makes a structure called a spore which is highly durable, enabling the bacteria to proliferate again when the environment becomes suitable for growth.

Furthermore, *Bacillus subtilis* is known to secrete antibacterial and antimycotic substances, and suppresses pathogenic and harmful microorganisms.

Used for microbial pesticide with low environmental burden and high level of safety

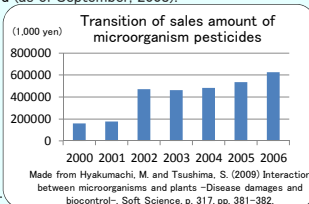
Plant diseases such as Gray mold of vegetables, fruits and flowers and powdery mildew of vegetables are caused by pathogenic fungi and have been causing great damages to agricultural products.

To solve this problem, a microbial pesticide using the antimycotic property of *Bacillus subtilis* was developed as a novel ecologically-friendly pesticide.



Among microbial pesticides, 8 sterilizing agents for sterilizing pathogenic and harmful bacteria and fungi are being sold (as of September, 2008).

Compared to chemical pesticides, microbial pesticides which use microorganisms from nature have merits such as less impact on the environment, high level of safety and less frequent emergence of resistance in pests toward the pesticide. Their sales have been increasing with expectations for their use in environmentally sustainable agriculture.



86. Examples of utilizing test strain (evaluation of antibacterial properties)

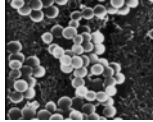
What is test strain?

Test strains are the strains of microorganism authorized to be used as the standard or reference in officially standardized tests such as those in ISO, JIS or the Japanese Pharmacopoeia.

Bacteria such as *E. coli* and *S. aureus*, which are indigenous to humans and may cause opportunistic infection, are often designated as test strains, and play important roles in quality control of industrial products.



Escherichia coli



Staphylococcus aureus

(National Institute of Technology and Evaluation (NITE), NBRG)

An example of antibacterial test

[Japanese Industrial Standards]

○JIS Z 2801: 2006

Antibacterial products – Test for antibacterial activity and efficacy (Amendment1)

A Japanese Industrial Standard which describes the test method and standard to judge if an antibacterial product has the required antibacterial effect.

Contributes to quality assurance of antibacterial products

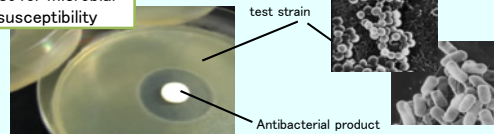
○Antibacterial product

Due to the people's interest in hygiene, the market for antibacterial products has recently been expanding greatly (860.3 billion yen (in 2003)).

To gain the people's reliability for these products, evaluation of antibacterial performance (efficacy) is indispensable.

Evaluation of antibacterial performance was made possible by tests using *Escherichia coli* and *Staphylococcus aureus*, and those tests are standardized in JIS and conducted by manufacturers or in test laboratories.

Test for microbial susceptibility



Evaluation of antibacterial performance

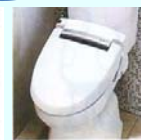
Commercialization



Antibacterial cutting board



Antibacterial mobile phone



Antibacterial toilet seat



Air cleaner equipped with antibacterial filter

References: Japan Food Research Laboratories (<http://www.jfrl.or.jp/item/effecttest/effecttest2.html>): Test for microbial susceptibility, Homepage of Society of International Sustaining Growth for Antimicrobial Articles: Antibacterial cutting board, antibacterial toilet seat
NCCX Inc.: Antibacterial mobile phone, Sharp Corporation: Air cleaner equipped with antibacterial filter

87. Examples of utilizing test strain (evaluation of microorganism contamination)

What is test strain?

Test strains are the strains of microorganism authorized to be used as the standard or reference in officially standardized tests such as those in ISO, JIS or the Japanese Pharmacopoeia.

Bacteria such as *E. coli* and *S. aureus*, which are indigenous to humans and may cause opportunistic infection, are often designated as test strains, and play important roles in quality control of industrial products.

An example of sterility test

[Japanese Pharmacopoeia]

4.05 Microbial limit test

Operating procedure and experimental conditions of the test to evaluate and judge the level of microbial contamination in non-sterile drug products, pharmaceutical ingredients, pharmaceutical additives, etc.

4.06 Sterility test

Operating procedure and experimental conditions of the test to judge the sterility of bulk drugs or drug products which are required to be sterile.

Contributes to safety assurance tests for medicine and medical equipment

○ Safety assurance tests for medicine and medical equipment (microbial limit test/sterility test)

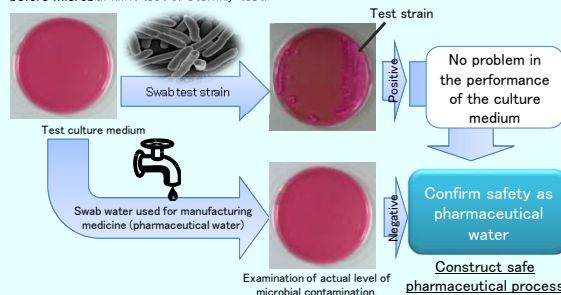
To assure the safety of medical products, it is necessary to examine the actual level of microbial contamination.

Verification of safety was made possible by tests using *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*, and *Pseudomonas aeruginosa*, and those tests are standardized in the Japanese Pharmacopoeia and conducted by manufacturers or in test laboratories.

Performance test for culture medium

A culture medium for growing microorganisms is used in standardized tests in the Japanese Pharmacopoeia, and its conformity to the test standard is important.

Therefore, a performance test for the culture medium using test strains is conducted before microbial limit test or sterility test.



Reference: Eiken Chemical Co., Ltd. Information magazine es (<http://www.eiken.co.jp/technique/es/pdf/es1.pdf>)

88. Examples of utilizing *Mycoplasma* (quality assurance of products)

What is *Mycoplasma*?

Mycoplasma is a genus of bacteria which is parasitic in the cells of eukaryotes. Since it is small enough in size to pass through a normal sterilization filter (0.22 μ m pore size), cell culturing media are often contaminated by *Mycoplasma*.

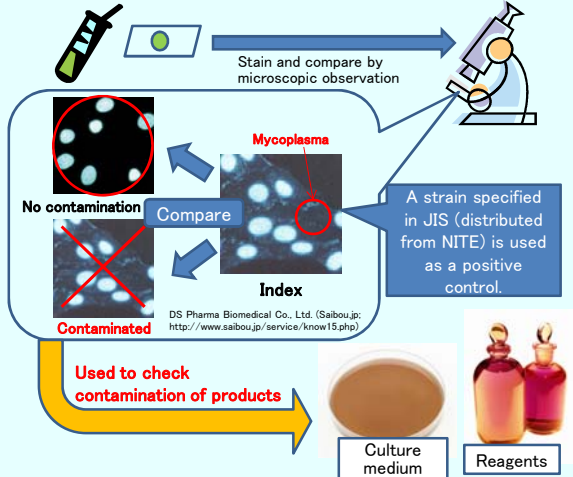
Japanese Industrial Standard (JIS) for detecting *Mycoplasma*

JIS is a set of standards for product quality or test methods related to manufacturing industry, and includes standards for inspecting *Mycoplasma* contamination.

e.g.
○ JIS K 3810-2:2006
Mycoplasma detection methods –
Part 2: Indirect DNA staining assay

Contributes to quality assurance of reagents etc.

Cultured cells, reagents for cell culturing and products derived from cultured cells are often contaminated by *Mycoplasma*. Therefore, contamination of *Mycoplasma* is tested using the strain distributed from NITE as a reference.



89. Examples of utilizing *Escherichia coli* (raw meat treatment)

What is *Escherichia coli*?

Escherichia coli is one of the major microorganisms existing in the environment, and resides in the digestive tract, especially in the large intestine, of birds and mammals.

Many strains of *Escherichia coli* are known and they are used as materials for various researches, as well as for the production of useful chemicals by genetic engineering.

On the other hand, pathogenic strains of *Escherichia coli* are also known.



(*Escherichia coli*)

Pathogenic *Escherichia coli*

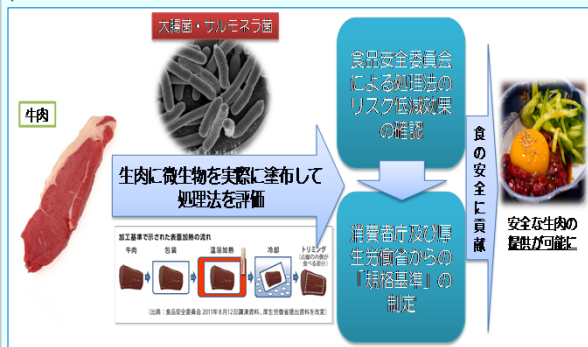
There are some strains of *Escherichia coli* which infect humans and produce toxins within the body such as the enterohemorrhagic *Escherichia coli* O-157, the causing agent for the mass food poisoning in Sakai-shi, Osaka in 2006, involving nearly 6,000 patients and even deaths mainly among elementary school students.

Contributes to safe supply of raw meat

In 2011, food poisoning by enterohemorrhagic *Escherichia coli* on beef occurred, due to the failure to implement proper treatment method of raw meat to avoid contamination by *Escherichia coli*.

Consequently, to ensure safe supply of raw meat, the Food Safety Commission of the Cabinet Office examined the effectiveness of raw meat treatment methods in reducing the risk of *Escherichia coli* and *Salmonella enterica* by actually swabbing these bacteria to raw meat.

The result was adopted by the Consumer Affairs Agency and the Ministry of Health, Labour and Welfare as the safety standard for edible raw meat (beef) pursuant to the Food Sanitation Act.



Reference: Cabinet Office Food Safety Commission (<http://www.fsc.go.jp/fscjis/survey/show/cho20120020001>)

90. Examples of utilizing *Salmonella* (rapid identification)

What is *Salmonella enterica*?

A species of bacteria living in animal intestines. Some are pathogenic, and cause food poisoning. It is known to cause gastroenteritis from infection such as via 24-hr circulating bath.



National Institute of Infectious Diseases

Detection of *Salmonella enterica*

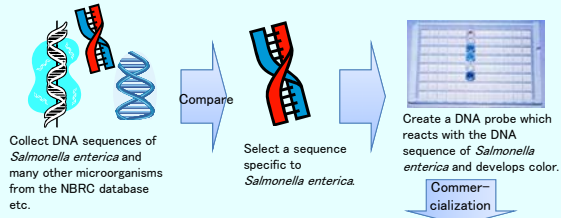
[Detection]

Since *Salmonella enterica* causes food poisoning, it must be detected at the scene of food processing. The method currently specified in JIS detects it from food samples by using a culture medium in which *Salmonella enterica* grows specifically. Since it takes about 4 days for the test result to come out, it is not suited for quick detection.

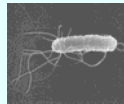


Developing quick identification kit for *Salmonella* using genome information

Search for a DNA sequence specific to *Salmonella enterica*. Make a "DNA probe" which develops color only when it reacts with the specific sequence. This enables quicker detection of *Salmonella enterica* compared to conventional methods.



Specific detection of *Salmonella enterica* became possible in 3 days even without expertise. Moreover, determining if a doubtful bacterial colony is *Salmonella* or not is possible in 3 hours.



Quick detection became possible.

National Institute of Infectious Diseases



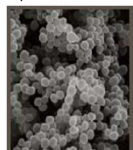
Kakusan Test (Scientific Feed Laboratory Co., Ltd.)

91. Examples of utilizing genome information (*Staphylococcus aureus*)

What is methicillin-resistant *Staphylococcus aureus* (MRSA)?

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major causative agent of nosocomial infections in hospitals worldwide.

Since effective anti-bacterial drugs are limited, it has been a great medical problem. Accurate and quick identification of MRSA has been a serious issue in clinical diagnosis and treatment of infected patients.



National Institute of Infectious Diseases
Evaluation (NITE, NBRC)
Staphylococcus aureus

Providing genome information of *Staphylococcus aureus*

As a result of comparing genome sequences of various MRSA and methicillin-susceptible *Staphylococcus aureus*, a DNA segment specific to MRSA was successfully identified.

Enables early diagnosis of MRSA infection

Uses the genome sequence specific to MRSA to develop a quick and reliable diagnostic method (patent).

Quick detection

→ Conventionally, detection had required 3 to 5 days, but it became possible in 3 to 4 hours!

1/30 of conventional method!

Simple method

→ The method and data are both simple, allowing anyone to detect, and the result is also easy to understand.

Anyone can detect with only a little training!

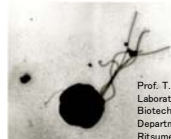
Early treatment of MRSA infection
Prevent spread of infection



92. Examples of utilizing genome information (anaerobic hyperthermophilic archaea)

What is *Thermococcus kodakaraensis* KOD1?

An obligatory anaerobic hyperthermophilic archaeon isolated from a solfatara in Kodakara-jima island, Kagoshima, which can grow at a temperature as high as 65°C to 100°C. Since the proteins produced by this archaeon are highly resistant to heat and are stable, their industrial utility is very high.



Prof. T. Imanaka,
Laboratory of Environmental
Biotechnology,
Department of Biotechnology,
Ritsumeikan Univ.

Providing genome information of *Thermococcus kodakaraensis* KOD1

The proteins of this archaeon are excellent in heat stability, and are expected to be used in industries such as chemicals and enzymes. Its DNA polymerase gene was identified from the genome information and is used for the production of the enzyme as a powerful research tool.

Production of reagents for molecular biology research

○ A DNA-replicating enzyme derived from a hyperthermophilic archaeon, *Thermococcus kodakaraensis*. It is highly accurate and fast to replicate DNA, and has been used widely.



Life Science Department,
Toyobo Co., Ltd.
(<http://www.toyobo.co.jp/bio>)

50 times more
accurate than the
conventional reagent*

Twice as fast as the
conventional reagent

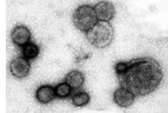
A must in the researches of genetic engineering

* Taq DNA Polymerase

93. Examples of utilizing genome information (influenza virus (1))

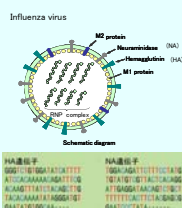
What is influenza virus?

- Human influenza is a serious viral disease which infects 10 million people annually in our country alone.
- There are 3 types of influenza viruses: A, B and C.
- Influenza virus genome changes rapidly, leading to the appearance of drug-resistant viruses and the emergence of new pandemic virus.



Picture provided by:
Influenza Virus Research
Center, National Institute of
Infectious Diseases

Providing genome information of influenza virus



Sequence analysis of key genes such as neuraminidase (NA) and hemagglutinin (HA) genes allows accurate typing of viruses and detection of variant viruses.

Predicting the annual epidemic strain of influenza

- It is necessary to correctly predict annual epidemic strains and select vaccine strains for the coming season well in advance.
- Therefore, genomic sequences of hundreds of viruses collected through local health institutes are analyzed, compared with the sequences of viruses which prevailed in the past, and vaccine strains are renewed annually.

Global activities to monitor human influenza virus



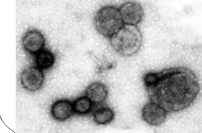
Influenza virus genome information analyzed by NITE contributed to the global monitoring activity of influenza virus.

Selecting vaccine strains for the coming season

94. Examples of utilizing genome information (influenza virus (2))

What is influenza virus?

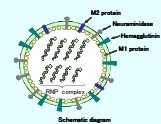
- Human influenza is a serious viral disease which infects 10 million people annually in our country alone.
- There are 3 types of influenza viruses: A, B and C.
- Influenza virus genome changes rapidly, leading to the appearance of drug-resistant viruses and the emergence of new pandemic virus.



Picture provided by:
Influenza Virus Research
Center, National Institute of
Infectious Diseases

Providing genome information of influenza virus

Influenza virus



Influenza virus genome comprises 8 RNA segments. By analyzing the entire genome, mutations which are suspected to contribute to higher virulence or drug resistance can be identified.

"An outbreak of pandemic influenza in 2009" Monitoring swine-origin novel influenza virus

- On April 17, 2009, the Centers for Disease Control and Prevention (CDC) confirmed the first case of "swine influenza" infection.
- Between April 28 and 30, WHO has raised the warning level from "Phase 4" to "Phase 5", and on June 11, to "Phase 6: pandemic".
- On May 9, infections were first identified in Japan at the quarantine (3 people at Narita).

Afterwards, NITE analyzed the genome of the influenza viruses found in domestic carriers, and submitted the analysis data to the National Institute of Infectious Diseases to contribute to the monitoring activity.

Targets of monitoring

◆ Change in antigenicity of the virus

The vaccines may lose effect due to variation in the region which determines the antigenicity.

◆ Increase in infectivity for human

Infectivity may increase due to variation in the region which is used to attach to human cells.

◆ Acquisition of resistance to Tamiflu and Relenza

These antiviral drugs may lose effect due to variation in the region which is the target of Tamiflu and Relenza.

◆ Transition to highly virulent type

Some regions are known to cause transition to a highly virulent type when variations occur.

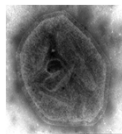
Monitor novel influenza, consider measures

95. Examples of utilizing genome information (aerobic hyperthermophilic archaea (1))

What is *Aeropyrum pernix* K1?

A hyperthermophilic archaeon sampled from a hydrothermal vent located at the shallow seabed of Kodakara-jima, Toshima-mura, Kagoshima, which has an optimum growth temperature of 90°C to 95°C.

Proteins and enzymes produced by this strain are highly stable at high temperature, and therefore are expected to be applied to industrial fields such as chemistry, food and medicine.



Aeropyrum pernix K1
(National Institute of Technology and Evaluation (NITE), NBRC)

Stability of thermophile proteins

- Since thermophiles live in high temperature, their proteins are heat resistant with highly stable structures.
- To elucidate functions of a protein, crystallization of the protein and analysis of its 3D structure are required, but crystallization of a protein is usually very difficult. **Proteins of thermophiles are easily crystallized, and are favorable for 3D structure analysis.**

Advance in genome analysis

- Due to the advance in genome analysis and identification of genes, protein expression using cloned genes became possible.

Used in a research which was awarded the Nobel Prize

☆ The Nobel Prize in Chemistry of 2003 was awarded to Roderick MacKinnon, M.D. of the Rockefeller University in the U.S. for his achievements in

Elucidation of functions of cell membrane channel proteins at an atomic level

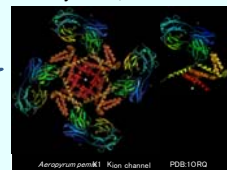
One of the achievements is

"X-ray structure of a voltage-dependent K⁺ channel"

Nature Vol. 423, No. 6935, pp. 33-41 (2003).

In this research, the channel protein was expressed using the genome information of *Aeropyrum pernix* K1, and the structure of the K⁺ channel was uncovered by X-ray crystallography. From this study, how K⁺ ions pass through the channel and why a smaller Na⁺ ion cannot pass through the channel were made clear. (Elucidation of ion channel mechanism will lead to remedies for neurologic and muscular disorders such as arrhythmia.)

Successful analysis
using easily crystallized
thermophile proteins.

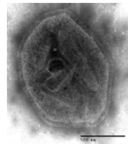


Cited from "Protein Data Bank Japan (PDBj)"
(<http://service.pdbj.org/mime/summary.j/1org>)

96. Examples of utilizing genome information (aerobic hyperthermophilic archaea (2))

What is *Aeropyrum pernix* K1?

A hyperthermophilic archaeon isolated from a hydrothermal vent at Kodakara-jima, Toshima-mura, Kagoshima. It optimally grows at temperature between 90°C to 95°C.



Aeropyrum pernix K1
(National Institute of Technology and Evaluation (NITE), NBRC)

Proteins produced by hyperthermophilic archaea are highly stable at high temperature, and therefore are expected to be utilized in industrial fields such as chemistry, food and medicine.

Discovering new knowledge in basic life science

An article, describing accurate gene assignment for *Aeropyrum pernix* genome, was published in a high level journal.

Contributes to improving accuracy of gene assignment

Number of citation: 34, related articles: 10
"Mol. Cell. Proteomics 2010 9: 415-426.", "Bioinformatics 2009 25: 1843-1845", "Microbiology 2009 155: 1758-1775.", "Bioinformatics 2009 25: 123-125", "Current opinion in microbiology 2009 12:292-300" ...

Providing genome information of *Aeropyrum pernix* K1

It has been commonly thought that a majority of genes start translation from a codon "ATG".

In *Aeropyrum pernix*, more than half of the genes are confirmed to start translation from an alternative codon "TTG", being a landmark discovery of life science.

Start codons of representative microorganisms

	ATG	GTG	TTG	その他
<i>Aeropyrum pernix</i> K1	28%	20%	52%	0%
大腸菌	83%	14%	3%	<1%
枯草菌	76%	22%	2%	0%
黄色ブドウ球菌	83%	7%	8%	2%
ビロリ菌	82%	10%	8%	<1%

○ Correct start codon of a gene

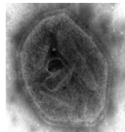
✕ Start codon of a gene predicted based on previous knowledge of biology

TTGGGAGCCGACTATCCGGGCCCTGTTAGT...GAG
... (omission) ...
GACATTATCGACCCAGAGGGCTTCGAGAGGCTGATGTCG
AGGCTTGGCGTAGAGAATGGGACCACGTCATACTCTAT
... (omission) ...
GTCGCAGTGTATGACGGGTCTTGGAGCGAGTGGGGCAAC
ATGGTCAGGGCTCCCGTTAAGAAGGGGACGAACCCTGA

97. Examples of utilizing genome information (aerobic hyperthermophilic archaea (3))

What is *Aeropyrum pernix* K1?

A hyperthermophilic archaeon isolated from a hydrothermal vent located at the shallow seabed of Kodakara-jima, Toshima-mura, Kagoshima. It optimally grows at temperature between 90°C to 95°C.



Aeropyrum pernix K1
(National Institute of Technology and Evaluation (NITE), NBRC)

Proteins produced by hyperthermophilic archaea are highly stable at high temperature, and therefore are expected to be utilized in industrial fields such as chemistry, food and medicine.

Providing genome information of *Aeropyrum pernix* K1

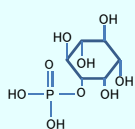
Many thermostable proteins derived from this archaeon have been used in researches and in industries. Its inositol-1-phosphate synthetase gene was identified from the genome information and is utilized for enzyme production.

Production of inositol-1-phosphate synthase (synthesis of B-group vitamin)

○ Inositol-1-phosphate synthetase derived from *Aeropyrum pernix*, a hyperthermophile archaeon, converts glucose-6-phosphate to inositol-1-phosphate. Since it is very stable and highly active even at 85°C, it enables efficient production of inositol-1-phosphate (a B-group vitamin).

Stable and active at 85°C

Efficient synthesis



Inositol-1-phosphate

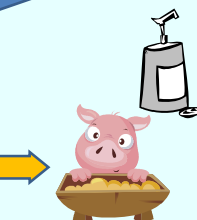
A B-group Vitamin

Possible involvement in anticancer effect?

Synthesis



Enzymes



Used in food, animal feed and cosmetics!

98. Examples of utilizing genome information (*Pseudomonas putida*)

What is *Pseudomonas putida*?

A bacterium mainly living in soils with various metabolic activities such as decomposing petroleum components which cannot be decomposed by other microorganisms.



Meijo Asian Research Center HP

It is expected to be applied in various industrial fields such as the decomposition of environmental pollutants, conversion of materials, and use in biocontrol agents. However, there is a related species which causes opportunistic infection, and some strains are suspected for their involvement in diseases.

Providing genome information of *Pseudomonas putida*

- Genomes of more than 200 strains of pathogenic bacteria have been analyzed, and the mechanisms of their infection and pathogenicity are being elucidated at the gene level.
- Especially, genome information of *Pseudomonas* has been published for as many as 60 strains, and many studies to evaluate the pathogenicity based on the genome information have been conducted.
- The rapid advancement in genome analysis technology, along with the efforts to organizing and utilizing genomic information, will enable reliable safety evaluation of microorganisms.

Safety evaluation based on genome information

Evaluation of safety based on the presence and absence of genes related to pathogenicity

△: Exists partly

病原菌に共通する 毒素・病原性遺伝子	病原菌		安全菌	
	<i>P. aeruginosa</i> PAO1	<i>P. aeruginosa</i> NBRC 12888T	<i>P. putida</i> KT2440	<i>P. putida</i> NBRC14841T
毒素生産	○	○	なし	なし
タンパク質分解	○	○	なし	なし
分泌	○	○	なし	△
クオラムセンシング (QS)	○	○	△	△
リパーゼ (LPS)	○	○	なし	なし
細胞取り込み	○	○	△	△
殺菌機構	○	○	なし	なし
表面粘性物質	○	○	なし	なし

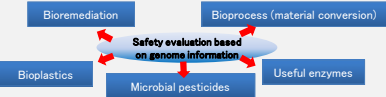
Note 1: A mechanism to perceive the cell density of the same species as its own and control the production of metabolites accordingly

Note 2: Enzymes which hydrolyze the ester bond in lipids

Toxin genes, genes for toxin export system, and other pathogenic genes were found in pathogenic species of *Pseudomonas* (*Pseudomonas aeruginosa*), however, none of such genes were found in non-pathogenic species of *Pseudomonas*.

<<Outcome>>

Rational safety evaluation of microorganisms becomes possible by accumulating and utilizing genome information of pathogens and related species, leading to the expansion of utilization range of microbial resources.



Used the results from "Project for safety measures in bioindustry", a commissioned study in FY 2011 of Ministry of Economy, Trade and Industry

99. Examples of utilizing genome information (classification of microorganisms)

Conventional classification methods of microorganisms

Classification and identification of microorganisms are indispensable for safe use of microorganisms.

Microorganisms have been classified and identified by morphological observation and chemical analysis, which require experience and expertise.

Providing genome information to serve as a reference for classification

The advancement of sequencing technologies enabled classification and identification of microorganisms based on DNA sequences of indicator genes. In the case of bacteria, classification and identification based on the sequence of 16S rRNA gene has become an accepted standard.

Simple and reliable molecular phylogenetic classification based on gene sequences

• Sequence of indicator genes

- Morphological observation
- Nutritional requirement
- ** metabolism
- ** bioactivity
- ...

Requires expertise



Anyone can do easily!

Outcome

Safety evaluation of microorganisms shifts from animal experiments to high resolution molecular phylogenetic classification.

The explanatory guide to the "guidelines for the bioremediation using microorganisms" was recently revised, and molecular phylogenetic classification based on gene sequences has now been recommended, as a replacement of animal experiments which used to be recommended for safety evaluation of microorganisms. Consequently, implementation of expensive animal experiments became unnecessary, lowering the hurdle for application drastically

