



Nagoya Castle

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Nagoya Castle, mentioned in Ise ondo (a Japanese traditional folk song) containing the famous line "Nagoya Castle is a very important landmark in Owari Nagoya," is known as one of the three great castles in Japan. It was constructed at the beginning of the 17th century by Ieyasu Tokugawa, who established the government of Edo after the Battle of Sekigahara. Before then, the center of Owari was at Kiyosu, with Kiyosu Castle being the center of government. However, the central government was transferred to present-day Nagoya in 1610 in order to prepare for the Toyotomi clan in Osaka and to serve as an important point on the Tokaido Route.

Many feudal lords were involved in the construction of Nagoya Castle, which became the residence of the Owari Tokugawa family, the head of the three branch families of the House of Tokugawa. Two of the most famous feudal lords were Kiyomasa Kato and Takatora Todo, who were not only master warlords but also master castle builders. Nagoya Castle brought to bear the most advanced castle construction technology of the age and served as a residence from the time of its occupancy by the 9th son of Ieyasu and the first local lord of the Owari, Yoshinao Tokugawa, until the 17th generation of the Owari Tokugawa family and the advent of the Meiji Restoration. The roof of the castle tower is decorated with a golden Shachihoko, symbolizing of the power of the castellan and giving the castle its nickname, Kinkojo (the castle with the golden Shachihoko). The golden Shachihoko is no longer just the symbol of the castle but has now also become the symbol of the city of Nagoya. Within the grounds of Nagoya Castle there are many structures, of which 31 have been designated as National Treasures. One is the

Hommaru Palace (the Main Palace) where the lords resided. This magnificent structure was built in the grand shoin style of traditional Japanese samurai residential architecture, covering approximately 3000 m² and containing over 30 rooms. It was decorated with luxurious and ornate pictures on partitions, including paintings by Kano artists such as Tanyu Kano. There were also decorative wooden panels with openwork carving above paper sliding doors as well as beautiful metal carvings created by the hands of master sculptors. This structure was known as the masterpiece of the castle.

Unfortunately, when Nagoya city was attacked during World War II in an air raid in May, 1945, most of the National treasures, including the Tenshukaku Tower and the Hommaru Palace, were burnt down. The remaining 6 structures and part of the painted partitions in the Hommaru Palace that escaped the fire are currently designated as important cultural properties.

The Tenshukaku Tower was reconstructed in 1959 and is widely known today as an important cultural feature of Nagoya. Furthermore, a project is underway to restore the Hommaru Palace, because 2010 will mark the 400th anniversary of the beginning of Nagoya government. The Hommaru Palace, which will be brought back to life through the use of both past and present technologies, will become a new cultural symbol for Nagoya and a cultural property of the world to be treasured and handed down to future generations.

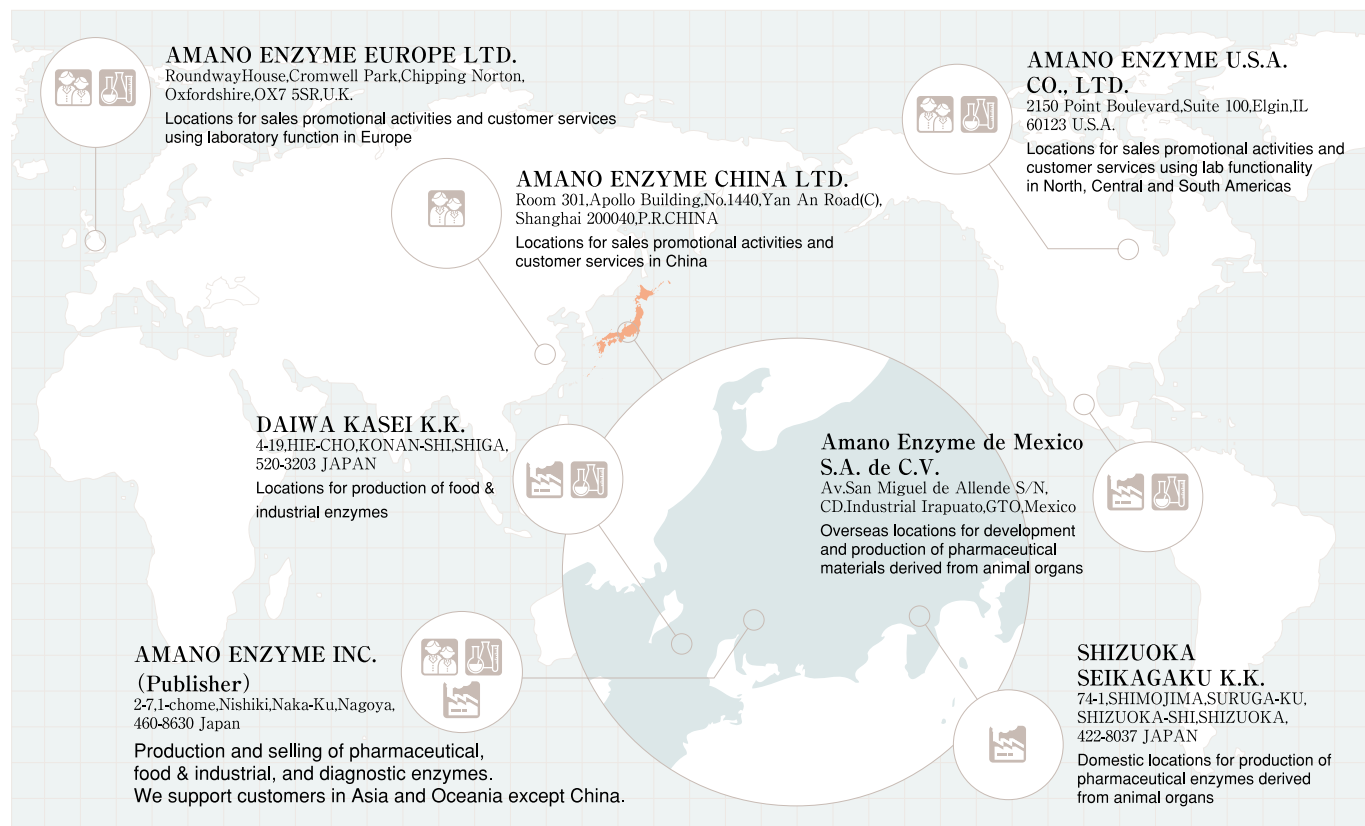


Reconstruction image of Hommaru Palace

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Aspergillus oryzae (koujikabi)

Aspergillus oryzae is often called *koujikabi* in Japanese. It is classified as an imperfect fungus, having no sexual state in its life cycle, and it is included in the genus *Aspergillus*. In Japan, the fungus was first isolated from rice *koji*, a preparation of steamed rice and *Aspergillus oryzae*, by a foreign educator, Hermann Ahlburg, who was employed by the Japanese government in the early Meiji period. As *koujikabi* could be easily grown on rice, it was initially named *Eurotium oryzae*, but it was later found to lack any sexual state and was therefore renamed *Aspergillus oryzae* (Ahlburg) Cohn. Because *koujikabi* was a useful fungus that predominates in *koji*, it was referred to as *koji-kin* to distinguish it from the other fungi in *koji*.

In Japan, *Aspergillus oryzae* (*koujikabi*) has been used for more than a thousand years in the making of such traditional fermented food products as *miso* (Japanese fermented soybean paste), soy sauce and *sake* (rice wine). The *sake* brewing method was the subject of a law code administered by the Imperial Court in the Nara period, and there is written records referring to *miso* in some, *Engishiki* (Code Law during the mid-Heian Period), dating from the Heian period. The use of the *Aspergillus oryzae* microorganism in the production of fermented food products therefore has a very long history. Eiji Ichishima, professor emeritus at Tohoku University, described the *Aspergillus oryzae* as *kokkin*, a national fungus of Japan, because so many traditional Japanese fermented food products could not have been made without it, and because it has had a great effect on all areas of Japanese history, society, economy, and culture. There is also a tradition in Japan of maintaining and controlling *Aspergillus oryzae* seed cultures used commercially by the fermentation industry so that it can be passed on from generation to generation. As a result, this fungus has been safely eaten as a food throughout its history and the food production method by *Aspergillus oryzae* has therefore been included in the GRAS (Generally Recognized As Safe) provisions of the United States FDA, since its safety has been confirmed worldwide.

The *Aspergillus oryzae* microorganism is also recognized as having contributed to the establishment of the modern microbial enzyme industry and its related technology, and the range of enzyme preparations derived from it can be described as "a treasury of enzymes". By using the *Aspergillus oryzae* to produce various enzymes, many different kinds of *Aspergillus oryzae*-derived enzyme preparations are now widely used.

Genome information and genome analysis

The term "genome" is used to describe the entire genetic information of an organism. The *Aspergillus oryzae* genome can be said to be its blueprint of life. The information regarding the numerous proteins and gene expression/regulation mechanisms that are needed to sustain the biological activities of *Aspergillus oryzae* are coded in the form of a sequence defined by four kinds of bases (ATGC).



Genome Structure of *Aspergillus oryzae* Machida et al., Nature, 438, 1157-1161 (2005)

Worldwide, genome analysis of many organisms commenced in 1990s and, in Japan, joint *Aspergillus oryzae* research teams were formed by industry-government academia to carry out EST analysis (analysis of the genes that are actually expressed) between 1998 and 1999. Since *Aspergillus oryzae* was such an important "national fungus", it was taken for granted that this work should be carried out in Japan. As a result, about 6,000 genes were obtained out of approximately 17,000 kinds of EST and, of these, about 49% were not homologous to any known genes and were therefore considered to be novel genes specific to the *Aspergillus oryzae*. Following on from this, a project to elucidate the entire *Aspergillus oryzae* genome sequence was then launched in 2001 by the DNA sequence center of the National Institute of Technology and Evaluation (NITE). This genome sequence analysis was carried out on a given sample of *Aspergillus oryzae* RIB40 using the whole-genome shotgun (WGS) sequencing method. A $10 \times$ genome sequence (about 10 times the size of the *Aspergillus oryzae* genome) was identified to link the DNA fragments obtained, the *Aspergillus oryzae* genome size of approximately 37 Mbp (37,000,000 base pairs) and 8 chromosomes and an estimated 12,000 genes (see the figure).

The characteristics of the Aspergillus oryzae genome

Almost simultaneously with the *Aspergillus oryzae* genome analysis, research groups overseas were engaged in genome analyses of *Aspergillus nidulans* (a genetic research strain) and *Aspergillus fumigatus* (a strain responsible for opportunistic infections). The results for genome size obtained were as follows: 37 Mbp for *A. oryzae*, 30 Mbp for *A. nidulans*, and 28 Mbp for *A. fumigatus*, while the corresponding estimated numbers of genes were 12,000, 9,300, and 9,000, respectively. These differences are considered to be due to the varied growing environments of the different *Aspergillus* strains.

When the genes for enzymes were examined and the estimated numbers of genes for proteases were compared, the total number was estimated at 135, 90, 99 respectively, the number of protease genes in *Aspergillus oryzae* was approximately 50% more than in other strains. From this, it may be deduced that having these many proteases may be a necessary requirement, considering the growing environment inhabited by the *Aspergillus oryzae* during actual brewing and fermentation applications.

What to expect in the genome analysis

Aspergillus oryzae is an important industrial microorganism used in food and fermentation engineering, and its fermented products are said to comprise "an arsenal of enzymes". As gene analysis for each enzyme has been carried out individually using recombinant gene technology, it has always been thought that elucidation of all the genes of *Aspergillus oryzae* enzymes would take a very long time and require a great deal of effort. However, with the genome information now on hand, all the information regarding the genes of known enzymes, as well as the genes of unknown enzymes, can now be obtained. The genetic information of *Aspergillus oryzae* has a great deal of industrial value, and it is expected that various useful enzymes with unprecedented characteristics may be present among the unknown genes in the genome of *Aspergillus oryzae*. In addition, almost no studies have yet been carried out for trace enzyme proteins detected only in small quantities in microorganisms. As more genome information becomes available, however, it is expected to search for these trace enzyme proteins based on genes and start an enzymological study. If such genes can be expressed in larger quantities, it will then be possible to analyze the functions of these trace enzymes resulting in the discovery and application of useful new enzymes.

1. What is a biorefinery

There is the concept of a biorefinery as a concretization of white biotechnology that has recently become increasingly important in Western countries. A biorefinery is a facility that systematically produces products such as valuable chemicals, bioethanol, and biodiesel through microbial fermentation of the sugar produced by decomposing biomass as a renewable resource. It is predicted that they will play a major role in a next generation of chemical industry instead of petroleum refineries that use crude oil to produce similar products.

2. The current status of and outlook for biorefineries

Because the starting materials used in biorefineries are mainly biomass-derived lignin, C5 or C6 sugars, the chemical products created differ from products manufactured in the current hydrocarbon-based oil refineries, and a new market is expected to emerge. According to the 2004 survey report entitled "Report on Biorefinery Research and Technology" prepared by the New Energy and Industrial Technology Development Organization (NEDO), the United States has defined the biorefinery concept as a key national strategy and is implementing a plan to replace 10% of all organic materials derived from petroleum with biomass by 2020, and 50% by 2050. In the United States, companies that are actively engaged in the development of biorefineries, such as DuPont or Cargill Dow, have linked up with the government to implement a long-range strategy.

Following the United States lead, European countries also delivered a road map of a biofuel technology platform up to 2030, centered on the European Union (EU), in 2006. The strategy serving as guidelines for the European Biofuel Technology Platform starts with the development of existing biofuels such as ethanol derived from grain starch and biodiesel derived from vegetable oil or waste oil, then lignocellulosic biofuels, and ends up with the establishment of comprehensive biorefinery systems. Compared to the United States, less information on products other than biofuels is available in European countries, but there are many companies, such as BASF, already equipped with a high standard of white biotechnology and therefore with much potential for further development. For this reason, it seems likely that they will become deeply involved in research and development of biorefineries.

In Japan, the activities of the Research Institute of Innovative Technology for the Earth (RITE), and the establishment of the Bio-fuel Technology Innovation Conference, led by the Ministry of Economy, Trade and Industry (METI), the Ministry of Agriculture, Forestry and Fisheries (MAFF) and 16 private companies, can be seen as evidence of progress. According to a report by Fusion & Innovation Inc., biorefineries will create a large market in Japan, whose size is estimated at 5,000 to 10,000 billion yen in the long term. In addition, the transition to an environmentally sustainable society will also be an important issue to neighboring Asian countries whose economies are rapidly growing such as India, China, and East Asian countries. Since Japan has always performed strongly in the areas of fermentation industry and technology, Japan may play a very large role in establishing biorefinery systems.

In order to avoid competition with food production, there is a global trend toward biorefineries using lignocellulosic biomass instead of grain starch-based biomass as a feedstock. Future issues requiring immediate attention are (1) to secure lignocellulosic biomass supplies while maintaining a good balance between our daily living activities and nature, (2) to establish efficient production technologies ranging from saccharification of lignocellulosic biomass to production of valuable chemical products using microbial fermentation of lignocellulosic biomass, and (3) to establish comprehensive biorefinery technology including techniques for efficient utilization of refractory biomass and fermentation residues.

Both the development of the economy through the creation of a new industry and market expansion and the resolution of global problems on the environment, energy, and food will be achieved by carefully promoting spreading of biorefineries that have emerged as a result of expectation for the realization of an oil-free, recycling-oriented society and recent advances in biotechnology such as post-genome technology.



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