Chapter 7

'Koji', a Mold, Plays the Most Important Role in Making Japanese Fermented Foods

The traditional fermented foods of Japan are characteristic in using *'koji'* (麹). The use of *koji* for the saccharification of starch contained in grains or soybeans is the most important for preparation of traditional fermented foods and seasonings in Japan. It is related to the climate of Japan with warm temperature and high humidity. Especially in the monsoon season, *tsuyu*, we can find many kinds of species of mold in our houses. You will be bewildered when you hear that *koji* is made from mold or fungi. So, I will explain the difference between Japanese traditional *koji* mold and common mold in nomenclature of the taxonomy. Of course, you can ignore the difficult scientific names of taxonomy.

7.1 Japanese *Koji* is a treasure of the world

The typical *koji* molds used in Japan include *Aspergillus oryzae* (Fig. 7.1 A and B), a filamentous yellow fungus, *A. sojae* (Fig. 7.2 B) used for soy sauce, *A. usami* and *A. awamori* var. *kawachi*, which are now classified into *Aspergillus luchuensis*, used for *shochu* (Fig. 7.1 C and B), and *A. awamori* (now classified into *A. luchuensis*) used for *awamori* (Okinawan rice spirit). *Aspergillus oryzae* (Fig. 7.1 A, B and Fig. 7.2 A), in particular, is a *koji* mold growing in Japan and it is used for making a lot of fermented foods such as Japanese *sake*, soy sauce (*shoyu*) and *miso*. *Aspergillus* is a name of a genus and *oryzae* is a name of a species. *Oryzae* was named after *oryza* which means rice. Mold used for Chinese fermented foods are mostly *Rhizopus* and *Monascus*, whereas wheat germ malt is usually used in order to brew whisky or beer in western countries. The malt contains enzyme, amylase, to degrade starch to glucose and maltose.

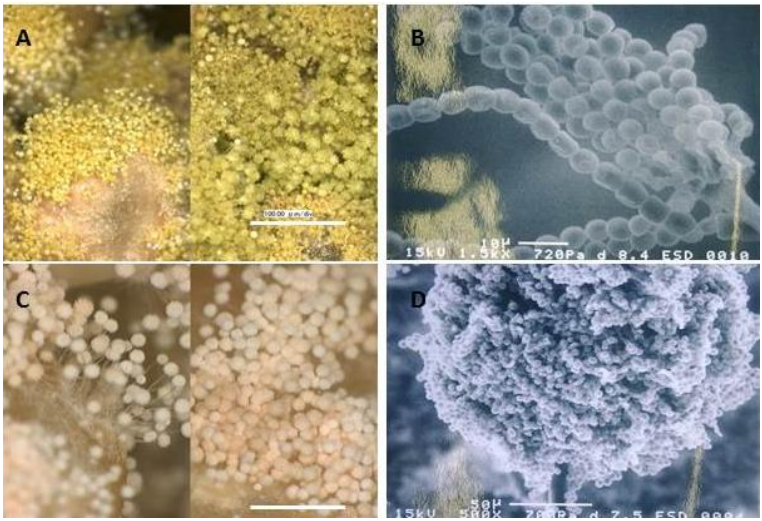

Fig. 7.1 *Microscopic and electron microscopic pictures of koji molds.*

A and B, *Aspergillus oryzae* (yellow *koji*) for sake brewing; C and D, *Aspergillus luchuensis* (white *koji*) for *shochu* brewing. A and C are microscopic pictures and B and D are electron microscopic pictures. White bars in A and C show 1 mm length, and those of B and D show 10 and 50 μm, respectively. (Pictures provided by Mr. M. Higuchi, Higuchi-Matsunosuke, Co. Ltd., Osaka, Japan)

Figure 7.3 shows that a man is splaying spores of *koji* molds on steamed rice placed on a large porous plate or cloth in a *koji–muro* (a *koji* room, 室(muro)). The temperature and moisture in the *koji-muro* are appropriately controlled for mold growth and the production of the enzyme. Spores of mold conidia (Fig. 7.2) germinate at about 32 ℃ to 36 ℃ (90~ 96 ℉) in high moisture and elongate mold threads (hyphae) in steamed rice grains. This sort of a workshop used to be seen in each *sake* breweries in Japan. However, these days, they don't work naked to the waist unlike former workers, however hot it is. After this work, they propagate *koji* mold in wooden containers that are piled up (Fig. 7.3) by controlling humidity and air.

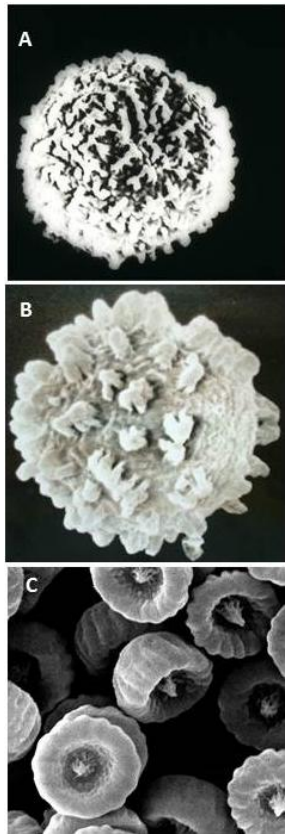

Fig. 7.2 *Electron microscopic pictures of koji mold conidia.*

A, *Aspergillus oryzae* (yellow *koji*) for *sake* brewing and *miso* production; B, *Aspergillus sojae* (yellow *koji*) for *shochu* brewing; C, *Aspergillus luchuensis* (black *koji*) for *awamori* brewing. The conidia are asexual spores which can germinate under an appropriate moisture and temperature. (Pictures provided by Mr. M. Higuchi, Higuchi-Matsunosuke, Co. Ltd., Osaka, Japan).

7.2 Can a computer take over the *Toji,* master of brewer?

Big *sake* breweries are making *koji* automatically by using fuzzy controlled computers instead of relying on experiences and intuition of the chief brewer (*toji*, (*toji,* 杜氏). Usually it takes time to become a skilled *toji* who has to master

brewing techniques including controlling of temperature and humidity and timing of mixing materials in *koji* making process and controlling of all the fermentation processes of brewing and maturation. That is why *toji* masters are usually elderly people, although we sometimes see young bio-engineers who are interested in the work of a *toji* recently. *Toji'*s work was done by men in the past, but these days the the work of *toji* seems to attract some young girls who feel it cool.

By the way, the store name of my ancestors was *Muro-ya*, and they were engaged in producing *koji* in one of the famous *sake* brewing towns, Saijo near Hiroshima. I majored in fermentation at my college days and I have been engaged in the research of this field. I sometimes wonder if my ancestors' DNA may be in my body.

7.3 Genomes of *Koji* mold designate the special feature of enzymes

Japanese scientists clarified that genome of *Aspergillus oryzae*, which is used for brewing of Japanese *sake*, rice vinegar, *miso* and soy sauce, is 125% as large as that of *Aspergillus nidulans* which is regarded as one of typical molds[*1]. They discovered it by determination of complete nucleotide sequences of the genomes (Fig. 7.4). Genomes mean total genetic information of each species of organisms. The genes of *A. oryzae* genomes which can be involved in the formation of secondary metabolites are 1,228 more than those of the latter species. Furthermore, *Aspergillus sojae*, which is also used in *shoyu* brewing, has 2,375 more genes than *A. nidulans.*

Fig. 7.3 *Preparation of koji in sake production process.*

To prepare *koji*, seeds (spores) of *koji* mold, *Aspergillus oryzae*, are sprayed on steamed rice (left) on a large porous cloth and the mixed rice is cultivated in a wooden container (right) through which temperature- and moisture-controlled air is passed to provide appropriate conditions for mold growth and the production of enzymes. Recently, however, a *toji* master does not work naked even if it is hot in the room. (Pictures provided by Mr. N. Miyashita, Yaegaki Syuzo Co. Ltd, Himeji, Japan.)

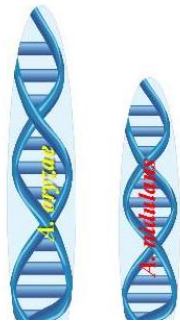

Fig. 7.4 *Comparison of genome size between koji mold, Aspergillus oryzae (left) and the type strain of fungus, Aspergillus nidulans (right).*

A *koji* mold produces a lot of several enzymes, such as amylases (Fig. 7.5) which break down starch into sugars, lipases (Fig. 7.6) which break down fat into fatty acids and proteases (Fig. 7.7) which break down protein into amino acids. We realized that a lot of degrading enzymes were produced by this larger genome than other mold genomes. In 1896 Dr. Jyokichi Takamine obtained a US patent for amylase production by a *koji* mold, *Rhizopus oryzae* (now known as *Aspergillus oryzae*) as the first Japanese scientist. He is known as a scientist

who developed a commercial product of gastrointestinal drug named 'Taka Diastase' made by *koji* mold at Sankyo Pharmaceutical Company. A lot of enzymes of *koji* mold work actively to degrade starch, lipids and proteins from rice and wheat grains and soybeans as ingredient for fermented products. *Koji* also makes original nutrients, which can grow yeast and lactic acid bacteria, and much physiological active materials like vitamins and amino acids.

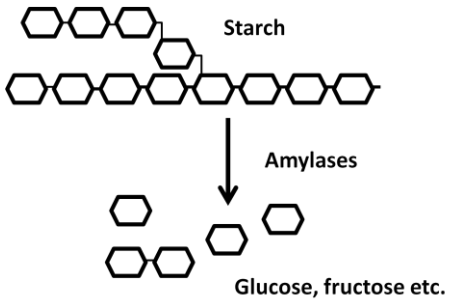

Fig. 7.5 *Degradation of starch to saccharides, such as glucose, maltose, etc. by starch degrading enzymes, amylases.*

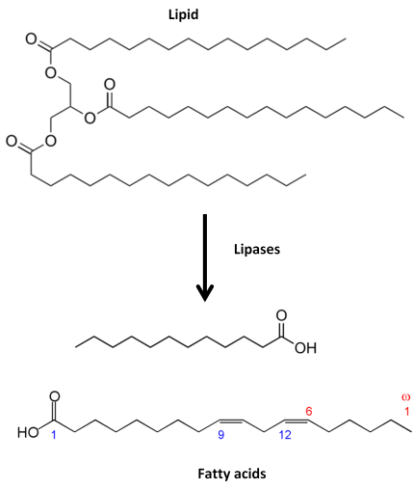

Fig. 7.6 *Degradation of lipids to fatty acids, such as palmitic acid, etc. by lipid degrading enzymes, lipases.*

Recently, salt *koji, 'shio-koji'* has become very popular as seasonings in Japan, so it will be paid attention to even in foreign countries in the near future.

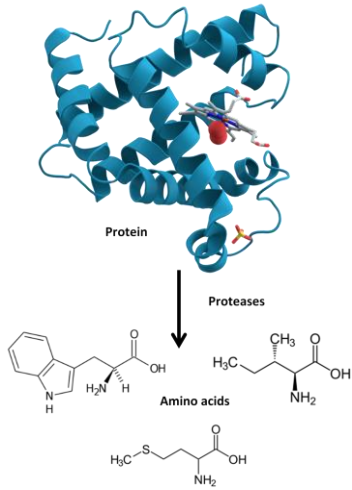

Fig. 7.7 *Degradation of proteins to amino acids, such as tryptophan, isoleucine, methionine, etc. by protein degrading enzymes, proteases.*

7.4 Japanese *Koji* does not make aflatoxin, a carcinogenic substance

Important information was revealed by the analysis of genomes of *koji* mold[*2]. It was that *koji* mold used in Japanese traditional brewing lacked part of the biosynthetic gene of aflatoxin which is contained in some molds, like *Aspergillus flavus* and *Aspergillus parasticus*. Aflatoxin takes a very strong part in carcinogenic activity even in very small amounts. Therefore, the customs are inspecting strictly whether or not imported rice or peanuts contain aflatoxin since rice grains or peanuts, which come from tropical areas, are sometimes infected by some molds (fungi) producing aflatoxin.

Scientists think that *koji* mold, *A. oryzae* has been changing gradually from *A. flavus* and lost a part of the aflatoxin biosynthetic genes either during the long history of its industrial use or from the beginning. Not only brewers but also we, Japanese people, feel at ease, now that *koji* mold is quite safe.

7.5 Summary

The *koji* is the most important for preparation of traditional fermented foods and seasonings in Japan. A lot of enzymes of *koji* mold work actively to degrade starch, lipids and proteins from rice, wheat grains and soybeans as ingredient for fermented products. *Koji* also makes original nutrients, which can grow yeast and lactic acid bacteria, and much physiological active materials like vitamins and amino acids. The genomic sequence analysis of *koji* mold demonstrated that the genome of *koji* mold is larger than that of a standard mold and lacks of the aflatoxin genes.

[*1] Machida, M *et al.*, Genomic sequencing and analysis of *Aspergillus oryzae*. Nature 438:1157-1161 (2005).

[*2] Machida, M., Yamada, O., and Gomi, K., Genomics of *Aspergillus oryzae*: Learning from the history of Koji mold and exploration of its future. DNA Research, 15:173-183 (2008).