Murakami H., 1980. “Koji-gaku” 麹学 (Comprehensive studies on *A. oryzae* based on koji fermentation), Brewing Society of Japan

An outline excerpted by the review authors

This book includes chapters describing about the origin and history of *koji* mold, *koji* seeds (or, *tane-koji* or *moyashi*) along with the strains suitable for their preparation, general features and preparation of *koji*, enzyme production and cellular constituents of *koji*, problems during fermentation, brewing with *koji* and *koji* industries, as well as other *koji*-related molds such as *Monascus* and *Rhizopus* (the contents referred to in the review article are mainly those described in the history of *koji*). *Koji* has been prepared with rice, wheat, soybean and other grains or cereals including wheat bran, obtained as a byproduct of wheat grain. The brewing of *sake* or related alcoholic beverages has been in close association with the rice-farming culture in Asian countries, in which starch grain is degraded and fermented by filamentous fungi into alcohol. Fungal species used for the fermentation depended on the grains used and the way they were processed. It appeared that *A. oryzae* was selected in Japan, because, unlike in other countries, steamed rice was used fermentation. Factors affecting the selection of fungal species and the cultivation method are discussed in detail by taking into consideration the ancient Chinese and Japanese historical literature. Use of leaf ashes was a key technology to purify *A. oryzae* for reliable fermentation, which has not been found in the continental Asia. The *sake* brewing technology including the preparation of *koji* was extensively developed in the 7th and 8th centuries and a detailed protocol for *koji* seed preparation for brewing *sake* for the Imperial Court was found in old literatures published in the 18th century. *Koji* was prepared and sold exclusively by a commercial association named *Koji-Za*, between the 12th and 16th centuries. However, the vested interest disappeared upon the emergence and growth of modern economy. The *sake* fermentation industry was further developed by the introduction of handicraft manufacturing in the 19th century. The *koji*-based fermentation technologies for other alcoholic beverages such as Japanese spirits called *shohu* and *awamori*, and for the preparation of *miso* (soybean paste), *shoyu* (soy sauce) and rice vinegar, as well as similar technologies developed for the preparation of Chinese alcoholic beverages are also described.

Murai T., 1989. “Tane-Koji Konjaku Monogatari” 種麹今昔物語 (The old and new story of *koji* seeds), in “Shushi-kenkyu” 酒史研究 (Studies on the Sake Brewing History), 7, 39-44.

An outline excerpted by the review authors

Prof. Sakaguchi, one of the renowned researchers in the fields of fermentation and brewing in Japan, who invented the so-called Sakaguchi flask (a flat bottom shaking flask with a shoulder for better aeration), once said that preparation of *tane-koji* (or, *koji* seeds) was the greatest invention in the *koji* fermentation. The *koji* industry was established in the Muromachi period (14th-17th centuries) through the development of reliable koji seeds selected by using leaf ashes. The *koji* seeds company named Kojiya Sanzaemon (糀屋三左衛門), in which the author of this book worked once as president, was honored to be the oldest industry in Kyoto: it was established 558 years ago. Since then and until 200-300 years ago, only two companies were licensed to produce *koji* seeds; one was callled *Kroban* (with the black emblem) and the other *Akaban* (red emblem). They distributed *koji* seeds to wide areas in the central part of Japan. In the more peripheral areas, people successively produced *koji* by adding small amounts of *koji* directly to raw materials. About 100 years ago, educated engineers developed the modern cultivation technology using an isolated microorganism, and the method was introduced into the manufacturing process at large soy sauce companies. The traditional method of preparation of leaf ashes for *koji* seeds preparation, different characteristics of *koji* seeds from the two major *koji* seeds distributors, differences in flavor, enzyme activities, length of hyphae and viability of *koji* during the production of *sake*, *miso* and soy sauce, etc. are described in detail. The company, Kojiya-sanzaemon, has been producing different types of *koji* seeds in different rooms under optimized conditions for each type of *koji* molds by complete avoidance of contamination of other microorganisms. Despite that the modern *koji* seeds industries with air-conditioned clean rooms and with computer-controlled processing, the author provided his company with traditional *koji* making rooms so that all staff of the company should work at least once a year by “talking with *koji*”.

Hata, Y & Ishida, H., 2000. "Glucoamylase-encoding genes of *Aspergillus oryzae* – Monograph –", Seibutsu-kogaku, 78, 120-127.

The English abstract

Aspergillus oryzae, the most commonly used fungus in the Japanese fermentation industry, is of considerable industrial significance due to its ability to secrete copious quantities of hydrolytic enzymes. Some hydrolytic enzymes – especially glucoamylases, proteinases, and phosphatases – important in fermentation processes are produced in much higher amounts in solid-state culture than in submerged culture. However, the mechanism of the solid-state culture of this organism, including its higher enzyme productivity, remains unclear. Here, we describe the cloning and characterization of two glucoamylase-encoding genes, *glaA* and *glaB*, and examine their expression in order to clarify the mechanism of higher glucoamylase production in solid-state culture of *A. oryzae*. Sequence analysis of the two gene revealed that the glucoamylase encoded by *glaA* consists of two functional domains, a catalytic domain at the N-terminus and a starch-binding domain at the C-terminus, as is the case other *Aspergillus* glucoamylases. However, the glucoamylase encoded by *glaB* has no C-terminal domain related to raw starch binding activity. The *glaB* gene is markedly expressed in solid-state culture, but only a little in submerged culture. To elucidate the mechanism of *glaB* induction in solid-state culture, we analyzed the promoter using the *uidA* gene (GUS gene) as a reporter gene. In solid-state culture, *glaB* expression at the transcriptional level is enhanced by low-Aw (water activity), high-temperature, and physical barriers to hyphal extension, as well as by starch. Deletion analysis of the promoter showed that substitution of the 12 bp GC-rich motif from -335 to -324 (GC box) resulted in significant loss of starch and low-Aw induction. These findings suggest that the GC bos is a cis-element essential for the high-level expression of *glaB* in solid-state culture.