Fish Fermented Technology by Filamentous Fungi

Journal of National Fisheries University 62 (4) 163－168 (2014)

Fish Fermented Technology by Filamentous Fungi

Tsubasa Fukuda, Manabu Furushita, Tsuneo Shiba and Kazuki Harada

Department of Food Science and Technology, National Fisheries University
2-7-1 Nagata-Honmachi, Shimonoseki 759-6595, Japan

Abstract : There is the greatest variety of filamentous fungal fermented food in Asia. *KOJI*, which is filamentous fungal fermented cereal, is used widely to seed or culture various fermented food and beverages such as miso, soy sauce, sake, and so on. *KOJI* is important enzyme producer, including amylase, protease, lipase, and so on. *KOJI* include not only filamentous fungi but also bacteria and yeasts. Effect of mixed culture in filamentous fungi fermentation are an improvement of fermented food nutrient and functionality and high efficiency of material conversion. Especially, a fermented fish products are often produced by filamentous fungi like *KOJI* because the use of KOJI on fish fermentation improves fermented products. Conclusions and future prospects are discussed.

Key words : filamentous fungi, *KOJI*, traditional fermented food, fermented fish

Introduction

A fermentation is often defined as the manufacture of foods employing the action of micro-organisms and their enzymes. It is a process in which complex compounds including carbohydrate, proteins, and fats are broken down to simpler forms. The products of fermentation have higher nutritional value than before. In addition, fermentation would ideally result in changes in the flavor, texture, color and other quality attributes that are considered desirable by the consumer.

Filamentous fungal fermentation is practiced in nearly all continents[1]. The number of fungal species encountered in fermented food is relatively limited, but they belong to various orders. Especially, the Asia has the greatest variety of filamentous fungal fermented food[2)3)]. Table 1 shows some major filamentous fungal fermented food in Asia. The reason is because that Asian climate which is higher humidity and temperature is optimum condition for the growth of filamentous fungi. Filamentous fungi are used for their enzymatic ability to degrade polymeric substances. They are important

Table 1 Major functional filamentous fungi in Asian fermented foods[2)3)]

Generic name	Natural Fermentation Product
Aspergillus	Koji (Japanese fermented cereals), Katsuobushi (Japanese fermented fish)
Penicillium	Katsuobushi (Japanese fermented fish)
Monascus	Ang-kak (Chinese red rice)
Neurospora	Oncom (Indonesian fermented groundnut)
Rhizopus	Tempe (Indonesian fermented soybean), Nuruk and Meju (Korean Koji), Chu (Chinese Koji), Bakhar (Indian Koji),
Mucor	Ragi (Indonesian Koji), Tempe (Indonesian fermented soybean)
Actinomucor	Sufu (Chinese fermented soybean)
Amylomyces	Ragi (Indonesian Koji)

traditional fish fermented foods in Asia.

Filamentous fungal fermented technologies have been artisanal in nature and have evolved by trial and error. For example, considerable amount of study has been done in the screening of useful microorganism. This article describes established filamentous fungal fermented technologies in Asia, the so called "*KOJI*" which is filamentous fungal fermented cereal. Moreover there are descriptions about fish fermented technologies by filamentous fungi.

KOJI: filamentous fungal fermented cereals

KOJI is used widely to seed or culture various fermented food and beverages such as miso, soy sauce, sake, and so on[2)3)]. *KOJI* is cooked cereal that has been inoculated with a fermentation culture or filamentous fungi. Thus *KOJI* consists of filamentous fungi and cereal. The Japanese word "*KOJI*" corresponds to "*CHOU*" in Chinese and "*NURUK*" in Korean. *KOJI* contains enzymes, such as amylase, protease, lipase, and many others. It is separated into two types according to their shapes in Asia: *BARA-KOJI* and *MOCHI-KOJI*.

a. *BARA-KOJI*[2)-4)]

BARA-KOJI is the Japanese type of *KOJI*. The shapes of *BARA-KOJI* is glandular. "*BARA BARA*" in Japanese means discrete (grains). *BARA-KOJI* is produced using a steamed cereal and *Aspergillus* strain. Rice, soybean and wheat are often used. The spores of *Aspergillus* strain are sprinkled onto the steamed cereal and left to cool and dry just a bit. This is then mixed up every couple of hours, so that at the end of the two-day *KOJI* propagation period, each and every one of the grains has the *Aspergillus* strain growing on its surface and into its center. *BARA-KOJI* is used for Japanese-sake, miso, soy sauce, and so on.

b. *MOCHI-KOJI*[4)5)]

MOCHI-KOJI is the other type of *KOJI*. The shapes of *MOCHI-KOJI* is block. *MOCHI* in Japanese means rice cake. *MOCHI-KOJI* is used in Asia except for Japan. *MOCHI-KOJI*. *MOCHI-KOJI* is produced using a raw cereals and *Rhizopus* strain basically. *MOCHI-KOJI* is produced by adding water to raw materials, cereal powders such as wheat, rice, and other starchy grains, and stiffening them by kneading and spores of *Rhizopus* strain and so on existing naturally adhere to and grow on it. Gochujang, Shao Xing Jiuare (a Chinese liqueur), and some fermented foods produced by using the *MOCHI-KOJI*.

Table 2 shows the summary of characteristics of *BARA-KOJI* and *MOCHI-KOJI*. In the process of *BARA-KOJI* fermentation, *Aspergillus* strains which had been stocked inoculated artificially. *MOCHI-KOJI* was fermented by environmental microorganisms under an open-system. *MOCHI-KOJI* consists of many kind microorganisms such as fungi, yeast, and some bacteria. Generally, *Rhizopus* strain is the principal microorganism of *MOCHI-KOJI*. For this reason, the fermentation period of *MOCHI-KOJI* fermentation is 4–30 days. In contrast, the fermentation period of *BARA-KOJI* fermentation is 1–3 days. Therefore the quality of *BARA-KOJI* is stabler than *MOCHI-KOJI*.

However, *MOCHI-KOJI* are used various culture substrates. The culture substrates are not only raw materials, grain powder such as wheat, rice, barley, but also acorn, and so on. If a characterization and physiological functionalities of *MOCHI-KOJI* develop, medical plants or herbs such as dandelion, chamomile, and acacia add. Thus, microorganisms in *MOCHI-KOJI* such as *Rhizopus* strains has a high ability of substrate utilization. In a recently study, *Rhizopus* strains produced useful materials (ex. enzyme, organic acids, etc.) from waste or unutilized resource[6)-8)].

Effect of mixed culture in filamentous fungi fermentation

Traditional fermented foods produced using natural (spontaneous) fermentation or mixed culture fermentation starters generally involve a number of microorganisms. These microorganisms grow in mixed

Table 2 Comparrison between BARA-KOJI and MOCHI-KOJI[2)-5)]

	BARA-KOJI	*MOCHI-KOJI*
Shapes	Galndular	Block
Culture substrate	Rice, soybean, wheat, etc.	Wheat, Rice, Barley, Soybean, etc. (Medical plant is often added.)
Preparation of culture substrate	Steamed	Raw
Fermentation period	1-3 days	4-30 days
Method for adding filamentous fungi	Inoculated	Natural drop
Principal filamentous fungi	*Aspergillus*	*Rhizopus*, *Mucor*, etc. (including various filamentous fungi)
Major country	Japan, etc	China, Korea, etc

culture in association with different microorganisms.

a. Improvement of fermented food nutrient and functionality

Rhizopus strains were necessary for the food fermentation such as *MOCHI-KOJI*, tempe (Indonesian traditional food), and so on. *Rhizopus* strain produce antibiotic in fermented food[9)]. The mixed culture of *Rhizopus* strain with *Bacillus* strain was increased production of antibiotic activity and altered the spectrum of activity relative to the each pure culture[10)]. The mechanism of increasing antibiotic activity is estimated that the production of the antibiotic from *Rhizopus* strain requires a step involving an enzyme that comes from the *Bacillus* strain[11)]. Actually, soybean fermentations using mixed culture of *Rhizopus* strain and *Bacillus* strain inhibited the growth of *Escherichia* coli.

Tempe is traditional Indonesian foodstuff produced by a solid fermentation of soybean with *Rhizopus* strain. Supplementation of the filamentous fungi inoculums with *Citrobacter* strain results in tempe enriched with vitamin B_{12}[12)]. Similarly, *Klebisilla* strain that produced vitamin B_{12} was used in a mixed culture with *Rhizopus* strain to produce tempe containing vitamin B_{12}[13)].

b. High efficiency of material conversion

It is difficult for *Rhizopus* strain to produce glucoamylase, which is important for the commercial production of glucose, from gelatinized starch under liquid culture medium. *Rhizopus* strain can produced a large amount of glucoamylase from using liquefied starch, which is produced by reacting α-amylase with gelatinized starch[14)]. It is well known that α-amylase is produced from *Bacillus* strain[15)]. Thus, mixed culture of *Rhizopus* strain and *Bacillus* strain produced at high levels of Glucoamylase production from gelatinized starch[16)].

Japanese rice wine is produced by the mixed culture of *Aspergillus* strain and other microorganisms (*BARA-KOJI*). Similarly, Chinese rice wine is produced by the mixed culture of *Rhizopus* strain and yeast (*MOCHI-KOJI*). The manufacturing process of this rice wine was simultaneous saccharification and fermentation. In addition, the metabolite of *Rhizopus* strains and *Aspergillus* strains gave the high tolerance against ethyl alcohol of yeast[17)18)]. For these reasons, the mixed culture system of filamentous fungus and yeast is the effective production method of ethanol.

Fish fermentation by filamentous fungi (*KOJI*)

Fermented fish products are often produced by filamentous fungi like *KOJI* because the use of *KOJI* on fish fermentation improves fermented products. *KOJI* is important enzyme producer, including amylase, protease, lipase, and so on. The fermented fish has a characteristic taste that develops from the auto-digestion of fish meat.

a. *Narezushi*

Narezushi, which is fish fermented together with rice or another starch, has a characteristic taste that develops from the auto-*difestion* of meat. Lactic and anaerobic bacteria proliferate, and yeast is also an important ingredient[19]. These act on the sugary content of the rice to produce many organic acids and alcohol. The effect of the organic acids is to lower the pH, controlling the growth of putrefying bacteria. Thus, *narezushi* can be preserved for long periods. *KOJI* is sometimes added *Narezushi* making process such as like *Kabura-zushi* (yellowtail sushi)[20]and *Izushi* (fermented pressed sushi)[21]. *KOJI* has strong capacity of glucoamylase production, which is important enzyme for the glucose production. Thus, organic acids and alcohol are produced effectively. All *narezushi* salt fish before the starch component is added. *Narezushi* is also considered as having originated from the technique of preserving fish by salting.

b. Fish source[22]

Fish sauces are traditional Japanese and Asian fermented food seasonings. In Japan, "*Shotturu*" is made from sandfish, "*Ishiru*" is made from squid, "*Ikanago-Shoyu*" is made from sand lance fish, and so on. In addition, "*Jeotgal*" in Korea, "*Patis*" in the Philippines, "*Nam pla*" in Thailand, "*Nouc mam*" in Vietnam and "*Yuiru*" in China are famous fish sauces in East and South Asia. Fish sauce is a clear liquid, straw yellow to amber in color, that has a mild cheesy flavor and fishy odor, a result of the slow fermentation of salted fish.

The fermentation is due to the proteolytic enzymes from the viscera as there are halotolerant. To accelerate fish sauce production, the process favors the shortening of hydrolysis and involves the use of *KOJI* prepared from *Aspergillus* strain inoculated in soybean.

Concluding remarks and future prospects

Filamentous fungi fermented products are used as *KOJI*. *KOJI* has high enzyme activities, including amylase, protease, lipase, and so on. Therefore, *KOJI* has a high ability of substrate utilization. A possible future development is the use of *KOJI* to prepare entirely novel products. In a recently study, *KOJI* produced useful materials from waste or unutilized resource.

Recently, various fish sources are made from unused fishery resources with *KOJI*[23)24)]. For example, puffer fish meat, whale meat, and sea urchin gonads. These resources are the processing residue from the seafood industry. The flavor of these fish sources using *KOJI* are different from the non-using *KOJI*, then the flavor of these fish sources using *KOJI* decrease the characteristic flavor of the non-using *KOJI*. However, these fish sources tastes good. Especially, the puffer fish sauce has a stronger antioxidative activity than the other fish and soy source[23)-25)]. Therefore, these fish sources have already been sold (Fig. 1).

Fig. 1 Novel Fish Source (Photos courtesy: Yamaka Shoyu Corporation in Japan)
Left: whale Middle: puffer fish Right: sea urchin

A great amount of *Kamaboko*, whose main ingredient is pasted whitefish, is are disposed as by-product or food processing residue. *Rhizopus* strain grew on *Kamaboko* like tempe. However, the smells of these fermented foods are musty and ammonia odors. To improve the scant of fermented *Kamaboko*, steamed rice was added because it is also possible to make combinations of fish meat and starchy grains so that the final fermentation product will have a more desirable nutritional balance. As a result, in the case that steamed rice mixed with *Kamaboko*, fermented mixture has pleasant smell like sweets or banana (Fig. 2).

The generation of organic wastes as pollutants is a global environmental problem. Fishery wastes or unuseful resources are produced in large quantities in Japan as a by-product and undersized fish. Most of these was simply dumped in the landfills or incinerated. Methods to convert these into value-added products are, therefore, highly desirable. Consequently, the filamentous fungi including *KOJI* have the potential to produce novel fermented fishes from unutilized resources. Further studies are needed to establish the detailed role of optimum condition. Moreover, we must investigate content of nutrition and bioactive compounds that are useful for health on this fermented food.

References

1) Ng TB: Fungal and Fermented Food. *In*: Fungal Biotechnology in Agricultural, Food, and Environmental Applications. CRC Press, , 223-231 (2003).

2) Hesseltine CW: A Millennium of Fungi, Food, and Fermentation. *Mycologia*, 57, 149-197 (1965).

3) Robert Nout MJ, Aidoo KE: Asian Fungal Fermented Food. *The Mycota*, 10, 29-58 (2011).

4) Ghorai S, Banik SP, Verma D, Chowdhury S, Mukherjee S, Khowala S: Fungal biotechnology in food and feed processing. *Food Res. Int.*, 42, 577-587 (2009).

5) Oba T: Koji of *Rhizopus* strain. *In*: Koji-gaku (in Japanese). Dai Nippon Printeing, Tokyo, 480-486 (1986).

6) Fukuda T, Sato T, Ishino Y, Tsutsumi K, Morita H: IGUSA waste: a novel substrate for Rhizopus strains to produce glucoamylase. *Jap. J. Food Eng.*, 9, 264-269 (2008).

7) Mass RHW, Bakker RR, Eggink G, Weusthuis RA: lactic acid production from xylose by the fungus Rhizopus oryzae. *Appl. Microbiol. Biotech.*, 72, 861-868 (2006).

8) Zhang ZY, Jin B, Kelly JM: Production of lactic acid from renewable materials by Rhizopus fungi. *Bioche. Eng. J.*, 35, 251-263 (2007).

9) Kobayashi S, Okazaki N, Koseki T: Purification and characterization of an antibiotic substance produced from *Rhizopus oligosporus* IFO8631. *Biosci. Biotech. Biochem.*, 56, 94-98 (1992).

10) Fukuda T, Yamamoto S, Morita H: Changes in the antibiotic production by co-culture or *Rhizopus peka* P8 and *Bacillus subtilis* IFO3335. *World J. Microbiol. Biotechnol.*, 24, 1893-1899 (2008).

11) Fukuda T, Tsutsumi K, Morita H: ANTIBIOTIC

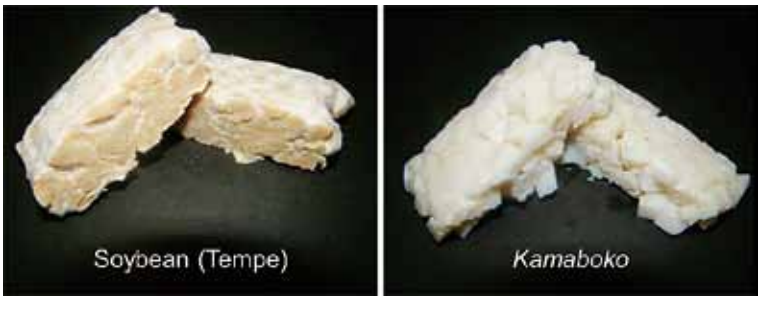

Fig. 2 Novel Fermented *Kamaboko* by *Rhizopus* strain
Left: fermented soybean (Tempe) Right: fermented *Kamaboko*

ACTIVITY IN CO-CULTURE: Influence of *Bacillus subtilis* on the Antibiotic Activity of *Rhizopus peka*. Jap. J. Food Eng., 9, 99–106 (2008).

12) Wiesel I, Rehm HJ, Bisping B: Improvement of tempe fermentaions by application of mixed cultures consisting of Rhizopus sp. and bacterial strains. *Appl. Microbiol. Biotechnol.*, 47, 218–225 (1997).

13) Nout MJ, Rombouts MF: Recent developments in tempe research. *J. Appl. Bacteriol.*, 69, 609–633 (1990).

14) Fujio Y, Morita H: Improved glucoamylase production by *Rhizopus* sp. A-11 using metal-ion supplemented liquid medium. *J. Ferment. Bioeng.*, 92, 554–557 (1996).

15) Gangadharan G, Nampoothiri KM, Sivramakrishnan S, Pandey A: Biochemical characterization of raw-starch-digesting alpha amylase purified from *Bacillus amyloliquefaciens*. *Appl. Biochem. Biotechnol.*, 158, 653–662 (2008).

16) Sato T, Fukuda T, Morita H: Glucoamylase Production in Submerged Co-Culture System of *Bacillu amyloliquefaciens* and *Rhizopus cohnii*. *Jap. J. Food Eng.*, 12, 55–63 (2011).

17) Kadokura T, Nakazato A, Takeda M, Chikubu S, Tanimura W: Influence of Culture Liquid of Fungi on Alcohol Tolerance of Yeast (in Japanese). *Seibutsu-kogaku kaishi*, 74, 167–170 (1996).

18) Kadokura T, Hatai S, Honda H, Nakazato A, Kaneko T, Takeda M: Influence of Culture Conditions or Rhizopus on Yeast Alcohol Tolerance in Rhizopus Culture Liquid (in Japanese). *Seibutsu-kogaku kaishi*, 85, 115–119 (2007).

19) Ishige N: Cultural Aspects of Fermented Fish Products in Asia. In: Fish Fermentation Technology. United Nations University Press, Tokyo, 13–32 (1993).

20) Kuda T, Yano T: Lactic Fermented Food with Fish (in Japanese). *Jpn. J. Food Microbiol.*, 27, 185–195 (2010).

21) Sasaki M, Kawai Y, Yoshimizu M, Shinano H: Effects of pre-pickling of salmon fillets on the chemical and microbiological characteristics of salmon *Izushi*. *Nippon Suisan Gakkaishi*, 71, 369–377 (2005).

22) Lee CH, Steinkraus KH, Reilly PJA: Fish Fermentation Technology. United Nations University Press, Tokyo, 13–32 (1993).

23) Harada K, Okano C, Kadoguchi H, Okubo Y, Ando M, Kitao S, Tamura Y: Peroxyl radical scavenging capability of fish sauces measured by the chemiluminescence method. *Int. J. Mol. Med.*, 12, 621–625 (2003).

24) Harada K, Maeda T, Hasegawa Y, Tokunaga T, Tamura Y, Koizumi T: Antioxidant activity of fish sauces including puffer (*Lagocephalus wheeleri*) fish sauce measured by the oxygen radical absorbance capacity method. *Mol. Med. Rep.*, 3, 663–668 (2010).

25) Harada K, Makino Y, Yamauchi T, Fukuda N, Tamaru M, Okubo Y, Maeda T, Fukuda Y, Shiba T: Efficacy of puffer fish (*Takifugu rubripes*) sauces in reducing hydroxyl radical damage to DNA assessed using the apurinic/apyrimidinic site method. *Int. J. Mol. Med.*, 20, 309–314 (2007).