Progress on the Breeding, Spawn-Making and Year-Round Cultivation of *Agaricus bisporus* in Fujian, China

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Abstract: This paper summarizes the progress on the strain improvement, spawn-making and year-round cultivation technology of *Agaricus bisporus* in Fujian, China and evaluates their impact on the development of the Chinese *A. bisporus* industry.

Key words: *Agaricus bisporus*, breeding, spawn-making, cultivation, mushroom industry

1 Introduction

In 2003, China produced over 650,000 tonnes of fresh mushroom and over 400,000 tonnes of processed *A. bisporus* products. China has become the biggest county worldwide for *A. bisporus* production and processing. Among the commercially cultivated strains, hybrid strain As2796 (bred by the Fujian Mushroom R&D Station of China) covered the largest cultivation area. This strain also accounted for 95% of all *A. bisporus* spawn consumption in Fujian province and over 80% within China itself. According to incomplete statistics, from 1989 until the present, As2796 has been cultivated over an accumulative total of 500,000,000 square meters, and produced about 4,000,000 tonnes of fresh white mushrooms. Therefore, As2796 has become one of the pre-eminent *A.bisporus* strains with the largest total output in the world. The spawn making technology currently adopted is based mainly on the bottled spawn technology used widely in Euramerican countries during the 1960s and 1970s. The spawn making technology for sterile, ventilated plastic bags, developed cooperatively by the Fujian Mushroom R&D Station (FMRDS) and the United States Unicorn Company, is now being introduced. The main cultivation technique is the double fermentation cultivation, seasonally based according to the natural climate from September or October until March or April the following year. However, the Chinese year-round cultivation technology developed in the recent years has made it possible to provide fresh white mushrooms to some city markets throughout the year. This paper will describe the history and progress of strain improvement, spawn making and year-round cultivation technology of *A. bisporus* in China (Fujian), and evaluate their promotion for the development of the Chinese *A. bisporus* industry.

2 The Import of Cultivation Strains and the Once-Fermentation Technique (1925-1977)

China lacks the germplasm of *A. bisporus*. Around 1925, the cultivation of *A. bisporus* was introduced into China from abroad. In addition to foreigners who built their small mushroom farms in Shanghai and Fuzhou, Changzhi Hu was the first Chinese person to introduce (from Japan) and cultivate *A. bisporus* in China. Zhinong Pang from Fuzhou, Fujian, began the domestic small-scale cultivation of *A. bisporus* in 1930, and Xiaotie Yu also began the cultivation at Hangzhou, Zhejiang Province in 1931. Around 1935, Shanghai began *A .bisporus* cultivation, the bedstead cultivation mode was popularized in the suburb in 1957, and in 1958 cow manure was successfully used instead of horse manure and the practice spread all over the country. In 1960, the Edible Fungi Institute of Shanghai was established under the auspices of the Shanghai Academy of Agricultural Sciences, and engaged in trials and studies on mushroom cultivation techniques including substrate formulation, mois-

ture management, diseases and pests control. The Institute also set up an *A. bisporus* cultivation mode suitable for the Chinese situation.[1,2] At the same time, Shanghai Normal University, Fujian Sanming Mycological Institute, Fujian Research Institute of Light Industry (FRILI), Zhejiang Agricultural University and the Institute of Food and Fermentation Industry of the Ministry of Light Industry began comprehensive studies on *A. bisporus* technology.

Fujian province began large-scale cultivation of *A. bisporus* 40 years ago. In 1966, large-scale cultivation and batch size processing were performed in Fuzhou, and 31 tons of mushrooms were exported in 1967. Subsequently, mushroom cultivation was developed in Xiamen, Zhangzhou, Quanzhou, Putian and Ningde, and the strains as well as the cultivation techniques were introduced from Shanghai. At that time, mushroom production in Fujian developed spontaneously without special organizations. In 1970, the province produced 1067 tons of fresh mushrooms, and this figure reached 10,000 tonnes within a short period. However, because of the disorganised use of strains, laggardly cultivation techniques and low yields, the annual mushroom production of the province from 1975 to 1978 remained stagnant at about 18,000 tonnes. During this period, the strains used in the mainland of China were mainly the old French strains, and were preserved by means of mycelium transfer, tissue isolation and multi-spore isolation. For cultivation, the main substrate was manure and straws, and the once fermentation technique was adopted. Thus, the production technology was behind, the scale was small and, although the mushroom quality was good, the output was low (the average yield was 3.6 kg/m^2).

In the 1977-78 production season, the output of *A. bisporus* was only 30,000 tonnes. Taiwan Province of China began to cultivate *A. bisporus* on a small-scale in 1953. In the 1977-78 season, with the application of the twice fermentation technique and the introduction of various varieties of *A. bisporus*, the yield per square meter reached 10 kg and the total output of *A. bisporus* reached 119,000 tonnes (the highest in history). In Taiwan, the white mushroom *A. bisporus* was mainly processed into canned mushrooms for export, which provided the Taiwan economic development with a large amount of foreign exchange. In this way, Taiwan became the third major producer of *A. bisporus* in the world.

3 Selection of Imported Strains and the Twice-Fermentation Technique (1978-1983)

From 1978 to 1979, Professor S.T. Chang (Fig.1), from the Chinese University of Hong Kong, recommended and provided to the mainland the twice fermentation technique and French high-yield strain 5-176. The Ministry of Light Industry built the Cooperation Network of Chinese Mushroom Research within a short time. Three meetings in the network were held at Fuzhou, Shanghai and Hangzhou in 1979, 1980 and 1981, respectively. The FRILI, the Institute of Food and Fermentation Industry of the Ministry of Light Industry, Zhejiang Agricultural University and the Edible Fungi Institute of Shanghai Academy of Agricultural Sciences were organized to take on the national "5.5" key project of mushroom science, "Studies on the breeding of excellent *A. bisporus* strains for canning and increased production" (1978-1983). China's mushroom researchers selected the introduced strains through comparison experiments and extended the twice fermentation technique, which contributed largely to the development of the white mushroom cultivation all over the country. The project obtained the 3rd National Science & Technology Advanced Prize of China. At that time, Fujian, Zhejiang, Guangdong, Jiangsu and Shanghai were the main producing areas in China. Fujian Province held the provincial mushroom production meetings in 1978 and 1981. The provincial government set up a special organization, the Fujian Mushroom R&D Station (FMRDS) in FRILI, to take charge of strain improvement and responsibility for the introduction of cultivation techniques, research, exploitation and popularization of the province. Branch stations were built in nine cities at the same time, so the provincial network for mushroom research and development was completed. Every year, the work meeting for mushroom spawn-making was held in Fujian Province to examine and approve the strains used in production, and so Fujian mushroom production was developed in an orderly way. In the 1982-1983 season, output of white mushroom in Fujian Province was 45,000 tonnes, representing 35% of the total production of China (130,000 tonnes), and yield rose to 5.0 kg/m^2. Meanwhile, the

annual production of Taiwan gradually began to fall. Production of *A. bisporus* in Taiwan was 56,800 tonnes whereas the yield reached 13.45 kg/m² during the same period.

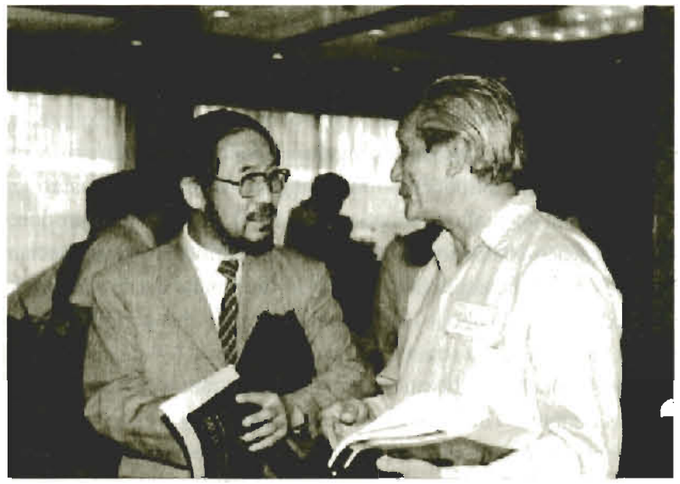

Fig 1. Prof. S.T. Chang and Prof. H.C. Wang of Fujian Research Institute of Light Industry exchanging views on mushroom technology

4 Selection of Spore Isolates from Strains and Amelioration of the Twice Fermentation Technique (1983-1988)

The central and local governments increasingly attached importance to the cultivation and processing of *A. bisporus* because of its great economic benefits and capability to earn foreign exchange. The cultivation of *A. bisporus* has been rapidly extending to areas south of the Yangtse River since 1983 and the main production areas have comprised ten provinces and municipalities, including Fujian, Zhejiang, Sichuan, Jiangsu, Guangdong, Guangxi, Anhui, Hunan, Jiangxi, and Shanghai. However, there were still some problems in production. Firstly, the spawn resource was disorganized and the strain character was not clear. Secondly, the twice fermentation compost technology could not be really extended because of restrictions in local energy sources and technology. Therefore, the Ministry of Light Industry of China again organized the FRILI, the Institute of Food and Fermentation Industry of the Ministry of Light Industry, Zhejiang Agricultural University, the Edible Fungi Institute of Shanghai Academy of Agricultural Sciences and Sichuan Research Institute of Light Industry to take on the national "6.5" key project of science and technology, "Studies on breeding of new *A .bisporus* strains for canning, increasing the production and canning technology" (1983-1985). Directed by Professor H.C. Wang, the team at FRILI embarked on breeding studies and improving cultivation technology. In China, the strains were selected by adopting the multi-spore screening method over 30 years, but no obviously improved strain remained. Thos should have been of concern for China since strain selection is one of the steps in spawn making. The strains were selected every year, and abandoned every year. Otherwise, the strains selected by the multi-spore screening method exhibited more genetic homogeneity than variability, in theory making it difficult to obtain obviously improved varieties. Compared with multi-spore screening, monospore screening has more potential for obtaining better strains. With great effort, extending over a three-year period, Z. C. Wang and his colleagues increased the average percentage of single spore germination from approximate 10% to above 60%. From among nearly a thousand single spore cultures isolated from the imported high-yield strains and good quality strains, they obtained 10 strains of *A. bisporus* with good properties. Out of the 10 strains, the "Min No.1" strain was widely cultivated both within and outside Fujian province. However, the strains from

single spore isolation inherited most of the characteristics of their parents. Therefore, the problem of high-yield strains lacking good quality and of good quality strains producing low yields still remained. After deciding that isozymes would make a suitable genetic marker, Z.S. Wang and his colleagues identified different strains, including homokaryotic strains and F1 hybrid strains, using gel electrophoresis. Based on the genetic analysis concerning the variable characters of strains, the method of crossbreeding by mating homokaryons to breed new strains was invented.[3-5] By this means, new strains of *A. bisporus* including As376, As555, As1671 and As2796 were obtained. Superior strains, accompanied by the new twice fermentation technique improved by J.Y.Ke, were popularized in Fujian province. Furthermore, researchers at FRILI were engaged in research work on strain preservation using liquid nitrogen. The cultivation of the white mushroom, *A. bisporus*, in Fujian Province began to advance along the road under the guidance of modern sciences. During the period 1987-1988, total production of *A. bisporus* in Fujian was 100,000 tonnes, amounting to 53% of the output of the whole country, and the average yield was close to 6kg/m². Meanwhile, mushroom production in Taiwan further decreased, and total production remained about 30,000 tonnes. During this period, Zhejiang Agricultural University, the Institute of Food and Fermentation Industry of the Ministry of Light Industry, and the Edible Fungi Institute of the Shanghai Academy of Agricultural Sciences also obtained great fruits from their mushroom-related research. The project was awarded the National 6.5 Science & Technology Key Task Prize in 1986.

5 Hybrid Strain As2 796, and Standardised Intensive Cultivation of *A. bisporus* (1989-2000)

In 1990, the Fujian Standard Synthesis for the Spawn and Canned Product of White Mushroom was approved by the Standard Bureau of Fujian Province, and the Fujian Byelaw for Mushroom Spawn Management was also approved by the Standing Committee of the People's Congress of Fujian Province. From 1990, the FRILI took out the intellectual property rights on the new hybrid strain, As2796, which together with the improved twice fermentation technique and standardized and intensive cultivation technology, comprised the new scientific model for *A. bisporus* cultivation in Fujian.[6-9] The enthusiasm of mushroom farmers was aroused by the improvement of the yield and quality of *A. bisporus*. In 1992, the Ministry of Light Industry held the 6th meeting of the Cooperation Network of Chinese Mushroom Research, and the participants visited the scientific growing model of Fujian Province. As a result, the standard and intensive cultivation technology was introduced to every production area, and was developed into several cultivation models. Sylvan Inc., the internationally well-known mushroom enterprise, collaborated with FRILI and jointly carried out research work on *A .bisporus*. Since then, China's white mushroom research has played a vital role in the international mushroom community. During the period 1995-1996, the production of *A. bisporus* in Fujian reached 279,000 tonnes, accounting for 75% of the output of China and 12.5% of production worldwide. The average yield rose to 9kg/m² and the highest yield reached 18kg/m². Fresh *A. bisporus* processed to canned mushroom and other products amounted to 220,000 tonnes. The production value of *A. bisporus* products was over 3 billion Yuan and the export value reached U.S.$200 million. Therefore, FRILI received the 2nd Science & Technology Advanced Prize of National Light Industry Ministry and 2nd Science & Technology Advanced Prize of Fujian Province. During this period, the white mushroom production of Taiwan amounted to only 5,000 tonnes.

During the 1996-1997 production season, the production technology and technicians of Fujian Province began transferring to the north of the Yangtse River, from Shandong, Henan to Xinjiang and the Northeast, developing new production areas and promoting the development of local mushroom production. As a result, the main area for mushroom production spread from the south to the north of the Yangtse River. Shandong, Henan and Hebei became the most promising young provinces in terms of mushroom production. Under the status, the FRILI took on the provincial "7.5" and the national "8.5" key project, "Study on the processing technology for white mushroom fresh-keeping", and opened a new consumption market for Fujian mushrooms. The market for fresh mushrooms in Fujian increased from 1000 tonnes in 1990 to near 50,000 tonnes, resulting in a stabilization of Fujian mushroom production. The yields of fresh mushroom were more than 10kg/m², even up to 20kg/m². In the

1999-2000 production season, total production of white mushrooms in China broke through 450,000 tonnes, taking first position in the world.

6 Genetic Breeding, Spawn-Making and Chinese Year-Round Cultivation of *A. bisporus* (2000-present)

By using gene engineering technology, special genes can be separated from a cell and transferred into another cell, which changes the genetic information of the receptor. It presents the breeding scientists with a beautiful future. In 1994, FRILI began to undertake research projects of the National Science Fund of Fujian Province and the Key Scientific Problem Tackling Programs of Fujian Province, and explored the theory of genetic engineering breeding of *A. bisporus* together with related breeding methods and experiments. Since then, more than two million Yuan has been spent establishing the genetic engineering laboratory that possesses a germplasm library of over 300 *A. bisporus* strains introduced from the world over, including strains with thermotolerance, dry-bubble disease resistance and green mould resistance. The laboratory, established in cooperation with Sylvan Inc., initially constructed the gene library of *A. bisporus*, and cloned some genes related to clustering and thermotolerance in *A. bisporus*.[10-12] In addition, Xiamen University, The Edible Fungi Institute of Shanghai Academy of Agricultural Sciences and Huazhong Agricultural University are also conducting molecular biological research on *A. bisporus*. strains improved by genetic engineering may soon be obtained.

Fig 2. In cooperation with a domestic colleague, the author collected wild *A. bisporus* strains from a meadow located in the Tibet altiplano of China

In China, 25,000,000 kg of spawn is needed every year for white mushroom production, most of which is hand-produced bottled spawn consisting of mycelia of different ages and which is inconvenient to use. To solve this problem, the spawn making technology for sterile, ventilated plastic bag was developed cooperatively by FRILI and the United States Unicorn Company. A corresponding mechanized production line was built, and the production procedure and quality control inspections introduced. Compared with bottled spawn, the production period for bagged spawn was shortened by 66% and the cost decreased by 32%. In addition, the bagged spawn was more convenient to use because the mycelia was of similar age and of good quality. This technology realized the innovation of spawn making for the white mushroom in China, and provided a strong base for year-round cultivation in China. By further reducing production costs, improving the spawn quality and yield, and increasing the scale of production, the FRILI will enhance the competitiveness of bagged spawn and enlarge its

market share, and gradually realize the professional production and distribution of white mushroom spawn.

Fig 3. Spawn is produced using ventilated plastic bags fitted with microbial filtration membranes

With the further development of the fresh white mushroom market in China, its year-round cultivation technology was also emphasized. Since the 1980s, China had introduced over ten mushroom production factories from Italy, the Netherlands and the United States, but most of them did not work normally because of high costs, inappropriate management or deficient techniques. At present, only the Jiufa Company of Yantai, Shandong is running well, and has become the first mushroom company to be listed on the Chinese stock market. Therefore, according to the Chinese situation, it is necessary to develop the Chinese model of year-round production technology. The FRILI undertook the provincial key scientific project, "Studies on the year-round cultivation technology of *A. bisporus*", which consists of three parts. The first part is to breed thermotolerant strains of *A. bisporus* and set up production bases at different altitudes using standard cultivation technology in order to provide fresh mushroom throughout the year (Figure 4). The second is to adopt strains with different temperature tolerance at a production base during different seasons to meet the year-round production. The third is to design the twice fermentation tunnel and construct a mushroom house that can maintain suitable temperatures and moisture levels for the industrial cultivation of *A. bisporus* in order to realize the year-round production of the white mushroom. The Chinese model year-round cultivation technology has been demonstrated and extended in Fujian Province.

The white *A.bisporus* mushroom, known as "health food" because of its wonderful flavor and rich nutrition, has been fashionable all over the world, and its consumption increases year after year. From 1925 to the present, the cultivation of the white mushroom in China, from none at all to just a few, from a few to the world's leader, has experienced a progressive process while its scientific research from import to digestion, from exploitation to creation, has steadily developed. In this way China has become one of the most advanced mushroom research countries in the world. Huge market demand and progress in science and technology indicates a brilliant future for the mushroom industry. Therefore we will exert our utmost efforts to create a still brighter future for China's mushroom industry.

Fig 4. Mushroom production bases set up in high altitude areas of Fujian province

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