KCS I-1

Study on the Genetic Diversity and Biological Characteristics of Wild *Agaricus bisporus* Strains from China*

Zesheng Wang[1], Jianhua Liao[1],, Meiyuan Chen[1], Bo Wang[2], Hongrong Li[1], Zhenghui Lu[1], Zhongjie Guo[1]**

[1]*Institute of Edible Fungi, Fujian Academy of Agricultural Sciences, Fuzhou 350014, P.R.China*

[2]*Research and Development Center of Edible Fungi, Sichuan Academy of Agricultural Sciences, Chendu 610066, P.R.China*

Abstract: 90 wild *Agaricus* strains from China, including 44 *Agaricus bisporus* strains identified preliminarily by isozyme electrophoresis, were studied by the techniques of SRAP and ISSR. 18 special SRAP bands and 12 special ISSR bands were analyzed, the strains were clustered and a demdrogram was obtained. The results showed that the strains were divided into 2 groups, wild *A. bisporus* group and the other *Agaricus* group. It is similar to the result of isozyme electrophoresis. 41 wild *A. bisporus* strains from Sichuan and Tibet were divided into 4 groups based on their growing places, suggesting the regionally difference of the strains to be quite obvious. Some white wild *A. bisporus* strains from Xinjiang and Tibet had special patterns, resulting in lower coefficient values with other wild *A. bisporus* strains. The biological characteristics of three wild *A.bisporus* strains were analyzed, and the results showed: 1. The wild strains grew slowly on PDA medium with weak appressed mycelia, and grew normally in kernel or fermented cottonseed shell substrate. 2. They grew faster than control strain As2796 under lower temperature of 16℃ and higher temperature of 32℃, with optimum growing temperature of 20-24℃, which was 4℃ lower than that of control strain. 3. In the cultivation with manure compost via twice fermentation, the mycelia grew normally in compost and quite slowly in casing soil, and the fruitbodies occurred less and late with easily opening and low production. 4. The fruitbody was off-white with flat and scaled cap, long stipe and dark gill. The bisporus basidia occupied 70-80% and trisporus basidia 20-30% of the total basidia. 5. Heterokaryotic monospore isolates could fruit in cultivation, and the homokaryotic isolates could cross with those derived from overseas wild *A.bisporus* strains. 6. The electrophoresis phenotype of isozymes such as esterase etc. belonged to high production type (H type). 7. The RAPD patterns made much difference from those of high

production, good quality or hybrid strains, which indicated that the wild strains produce a new kind of RAPD type.

Key words: *Agaricus bisporus,* Wild strains, Biological characteristics, Genetic diversity, SRAP, ISSR, China

Agaricus bisporus (Lange) Sing is the most widely cultivated and consumed edible fungi in the world with important economic value. In 2004 its world total production was near 3,500,000 tons, producing more than 10 billion of US dollars. The cultivation of *A.bisporus* began in France 300 years ago, and its spawn purification and preparation and strain improvement also had a history over 100 years. In about 1925 *A.bisporus* was introduced into China for cultivation, and in 1978 the strain improvement was started and the technology of compost twice fermentation was introduced. Since 1999, China had become the biggest country for *A.bisporus* production, and the hybrid *A.bisporus* strain As2796, which came from the crossbreeding of introduced germplasm, became one of the commercial strains producing largest annual yield of fresh button mushroom in the world [1]. However, the development of mushroom industry called for the strains with stronger resistance to environment and diseases, and the introduction of wild germplasm was one of the possible ways solving the problem. Wild *Agaricus* resources are widely distributed in China[2], which can be found from Liaoning, Neimenggu to Yunnan, Sichuan, Tibet and Xinjiang province. Therefore, China is also an important area for *Agaricus bisporus* distribution in the world. But in a long period the wild *A.bisporus* in China were not collected, identified and evaluated systematically, which limited the usage and development of the important resources[3,4,5]. In recent years, cooperated with Sichuan Academy of Agricultural Sciences, Fujian Mushroom R&D Station have paid much attention to the systematical collection and identification of Chinese wild *Agaricus* resources especially *A.bisporus* resources, built up Chinese wild *A.bisporus* germ plasm library, studied and evaluated their genetic characters, which would supply available domestic parent materials for strains improvement, and would be of important significance to the diversity and continuous application of world *A.bisporus* resources.

In this study, 90 wild *Agaricus* strains collected from China were analyzed by SRAP (Sequence-related Amplified Polymorphism) and ISSR (Inter-Simple Sequence Repeats) fingerprinting to get their similarity and supply DNA basis for wild *A.bisporus* identification, and biological characteristics researches of part of wild isolates were reported.

1. MATERIALS AND METHODS

1.1. Materials

1.1.1. Strains: 90 wild *Agaricus* strains used in DNA fingerprinting were collected from Tibet, Xinjiang, Ningxia, Sichuan, Qinghai and Gansu province of China. They were all regarded as *Agaricus* strains based on their fruitbodies phenotypes and growing environment, among which 44 *Agaricus bisporus* strains were identified preliminarily by isozyme electrophoresis Three wild *Agaricus* strains used in biological characteristics analysis, Ag4-3, AgS4 and Agm7, were collected from the meadow of Tibet altiplano and identified as *Agaricus bisporus*. All the strains above and the control strains used in this study were supplied by the Institute of Edible Fungi of Fujian Academy of Agricultural Sciences.

1.1.2. Media: General PDA medium, kernel medium, fermented cottonseed shell medium and fermented manure compost were used in this study.

1.1.3. Primers: 6 pairs of SRAP primers (me1-em2, me1-em5, me2-em3, me2-em4, me5-em9, me5-em10) and 2 ISSR primers (808,809) used in this study were selected by Chen et al. in the DNA fingerprinting of 206 cultivation strains of *A.bisporus*[6], and their sequences were cited from references[7,8].

1.2. Methods

1.2.1 Mycelia culturing, total DNA extraction, SRAP and ISSR analysis were the same as reference [6], and the software NTSYSpc-2.02j was used for clustering analysis.

1.2.2. Growing temperature test: The strains cultured under 24℃ for several days were transferred respectively to different temperature of 16℃、20℃、24℃、28℃、30℃、32℃ for culturing, with three repeats for each treatment, and the growth speed of mycelia was measured.

1.2.3 The isolation, cultivation and identification of heterokaryon and homokaryon monospores of wild strains, as well as the homokaryon crossbreeding and fruiting test were described before [9].

1.2.4 The mycelia isozyme Polyacrylamide gel electrophoresis (PAGE) and patterns analysis of esterase (Est), polyphenol oxidase (PO), cytochrome oxidase (COD) and peroxidase (POD) were based on the methods described before [10,11].

1.2.5 The RAPD analysis of mycelia total DNA was described before [12].

1.2.6 Basidia observation under microscope: The gills separated from mature fruitbodies were observed under Olympus phase contrast microscope (400X).

2. RESULTS AND ANALYSIS

2.1. Genetic diversity of 90 Chinese wild *Agaricus* strains

2.1.1. DNA fingerprinting of 90 Chinese wild *Agaricus* strains

The total DNAs of 90 wild *Agaricus* strains from China were analyzed by SRAP and ISSR. 18 special SRAP bands were found in the patterns amplified by primer pairs of me1-em2, me2-em4 and me5-em10, and 12 special ISSR bands were obtained with primer 808 and 809. Part of the amplification results were showed in Fig.1-Fig. 4.

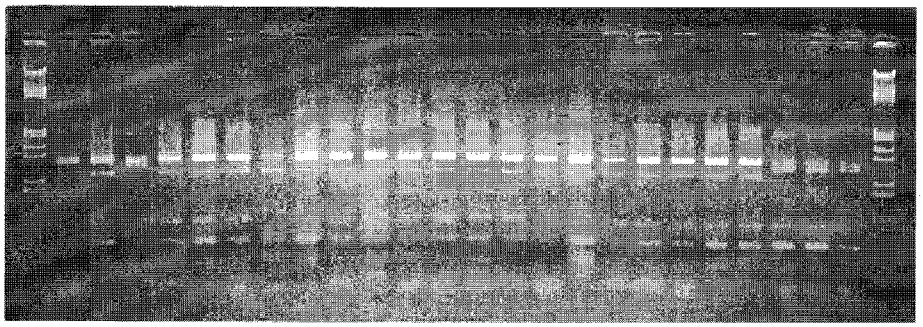


Fig. 1. SRAP patterns of 24 wild *Agaricus bisporus* strains from China (primer pair me5-em10) The first and last lane: LambdaDNA/EcoRI+HindIII Markers, similar here in after.

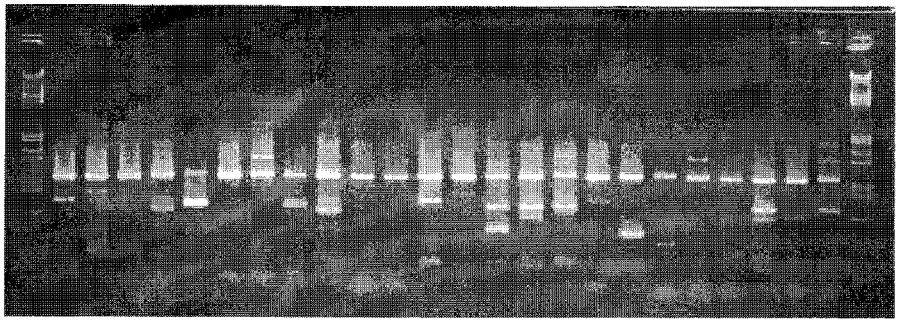


Fig. . SRAP patterns of 24 wild *Agaricus* strains from China (primer pair me5-em10).

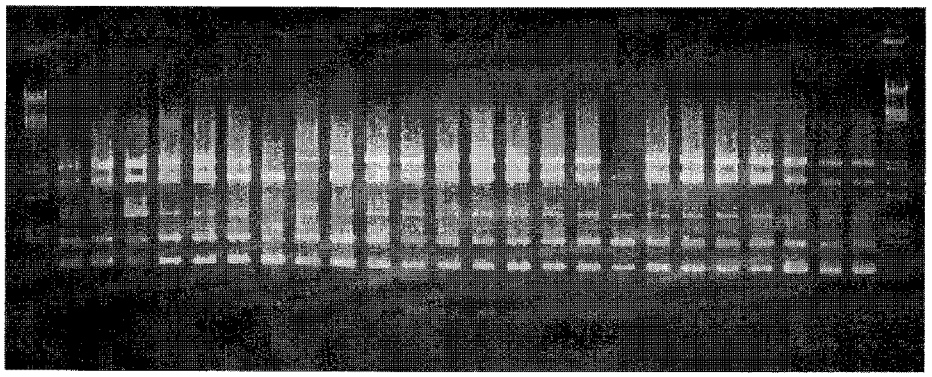


Fig. 3. ISSR patterns of 24 wild *Agaricus bisporus* strains from China (primer ISSR809).

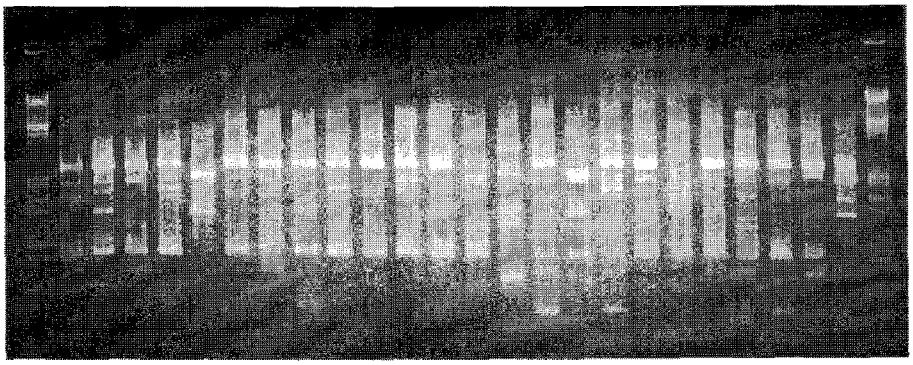


Fig. 4. ISSR patterns of 24 wild *Agaricus* strains from China (primer ISSR809).

2.1.2. Cluster analysis of 90 Chinese wild *Agaricus* strains

According to the 18 special SRAP bands and 12 special ISSR bands above, 90 Chinese wild *Agaricus* strains were analyzed and clustered, and a demdrogram was obtained (Fig. 5). The results showed that the strains were divided into 2 groups, wild *Agaricus bisporus* group and the other *Agaricus* group. It is similar to the result of isozyme electrophoresis. 41 wild *Agaricus bisporus* strains from Sichuan and Tibet were divided into 4 groups based on their growing places, including 1 group of Lhasa Tibet, 2 groups of Naqu Tibet and 1 group of Hongyuan Sichuan, which suggested that the regionally difference of the strains were quite obvious. Among the groups there was relative higher genetic similarity over 62% because of their characters of wild *A.bisporus*. The strains in each group had similar band patterns, except that 2 strains in Lhasa Tibet group, Ag78317 and Ag781313, made a little difference from those of other strains in the same group. Some white wild *Agaricus bisporus* strains from Xinjiang and Tibet, i.e. Xinjiang Wild, AgX04 and AgX042 had unique band patterns, resulting in lower coefficient values of 10% -13% with other brown or light brown wild *Agaricus bisporus* strains. Except for *A.bisporus*, the other *Agaricus* strains, most of which was collected from Gansu or Qinghai province, had a wide genetic diversity. Their genetic similarity changed from 18% to 100%, and most of the strains had their own special band patterns and were separated exactly on the dendrogram.

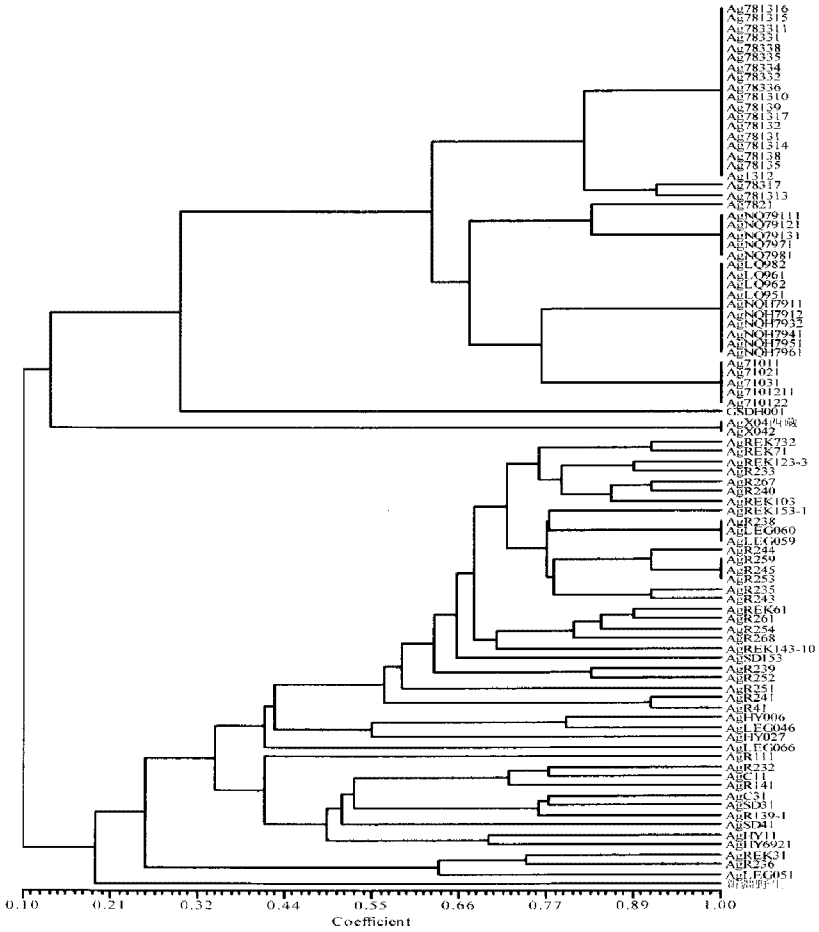


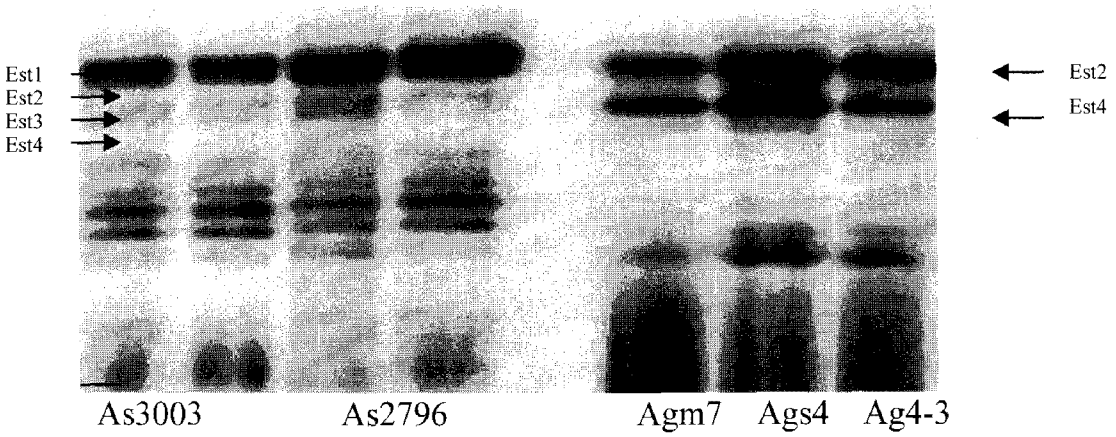
Fig.5 Dendrogram of 90 Chinese wild *Agaricus* strains

2.2. Biological characteristics analysis of strain Ag4-3, AgS4 and Agm7

2.2.1. The culturing results showed that the wild strains grow slower than control strain As2796 on PDA medium with weak and appressed mycelia, but their growth speed was close to that of control strain As2796 in kernel medium. Three wild strains grew faster than control strain As2796 in fermented cottonseed shell compost under lower temperature of 16℃ and higher temperature of 32℃, with optimum growing temperature of 20-24℃, which was 4℃ lower than that of As2796. The results indicated that the wild strains have better ability of low and high temperature tolerance.

2.2.2. The mycelia isozyme electrophoresis phenotype of esterase, polyphenol oxidase, cytochrome oxidase and peroxidase of the three wild strains all belonged to high production type (H type). The esterase isozyme had stronger activity, resulting clear bands and stable patterns (Fig. 6).

+



Special bands for HG4 type: Est1、2、3、4 Special bands for H type: Est2、4

Fig. 6. Polyacrylamide gel electrophoresis (PAGE) patterns of esterase isozyme of wild strains mycelia

2.2.3. The mycelia total DNA of three wild strains were extracted and RAPD experiments were carried out by using random primer H4, H5, U16, U18, U20, B1 and M7. The results showed that the three strains have similar RAPD phenotype, but which made much difference from those of high production strains (02, 176, 111), intermediate type strains (LMi, SA6), good quality strains (Fuguan4, 8211 , 8213) and hybrid strains (As3003, As2796), and belonged to a new RAPD phenotype. Fig.7 and Fig.8 showed the RAPD patterns of primer M7 and U18.

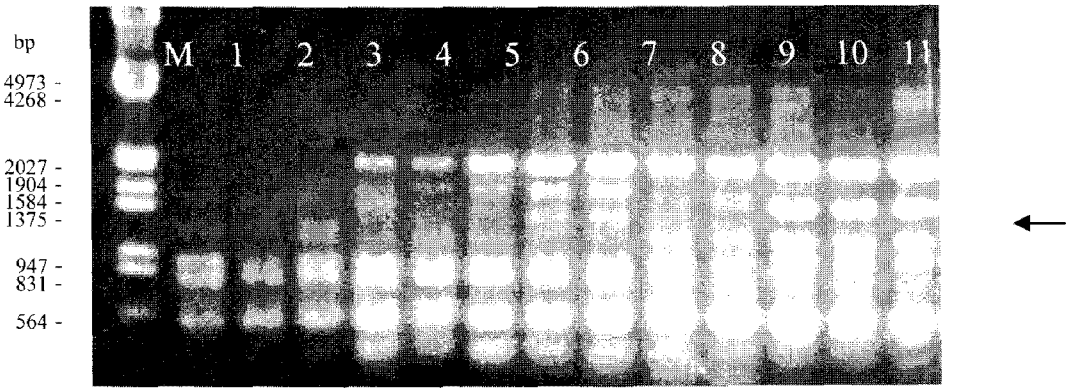


Fig. 7. RAPD patterns of random primer M7.

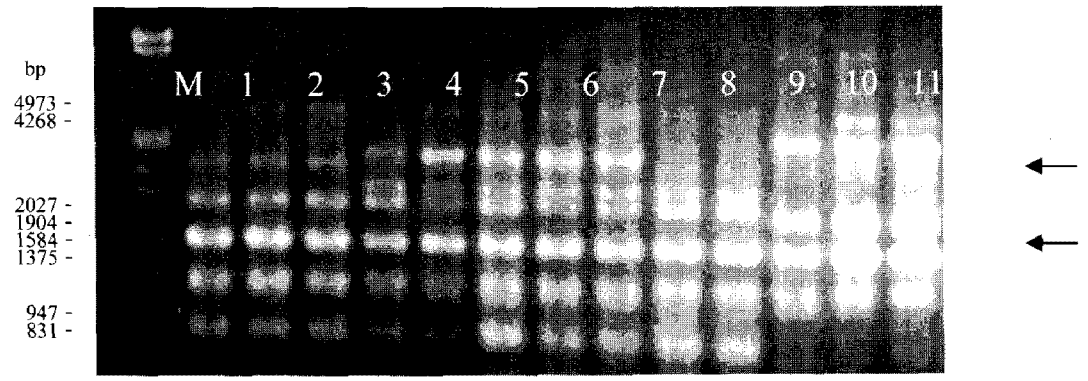


Fig. 8. RAPD patterns of random primer U18.

Lane M: LambdaDNA/EcoRI HindIII markers, Lane 1-13: 02, 176, 111, LMi, SA6, Fuguan4, 8211,8213, As3003, As2796, Agm7, AgS4, Ag4-3. The arrows show the special bands of wild strains.

2.2.4. Fruiting test: In the cultivation with manure compost via twice fermentation, the mycelia germinated and grew normally in compost and quite slowly in casing soil, and the fruitbodies occurred less and late with easily opening and low production. The fruitbody was off-white with flat, thin, scaled and centrally concaved cap, long and straight stipe, velum-like annulus and dark gill (Fig. 9, Fig. 10).

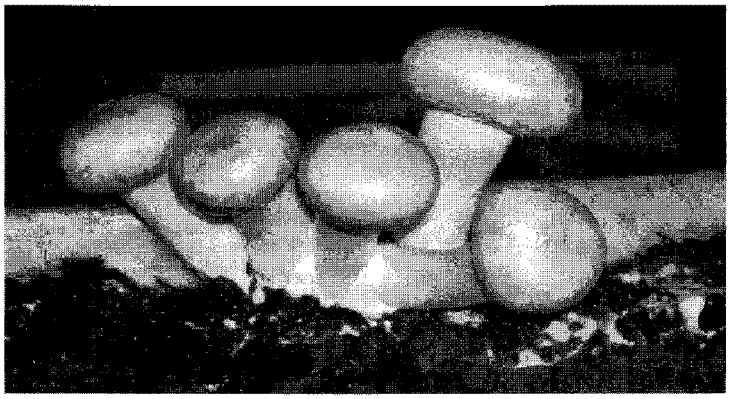


Fig. 9. Fruitbodies in manual cultivation of wild *A.bisporus* from Tibet.

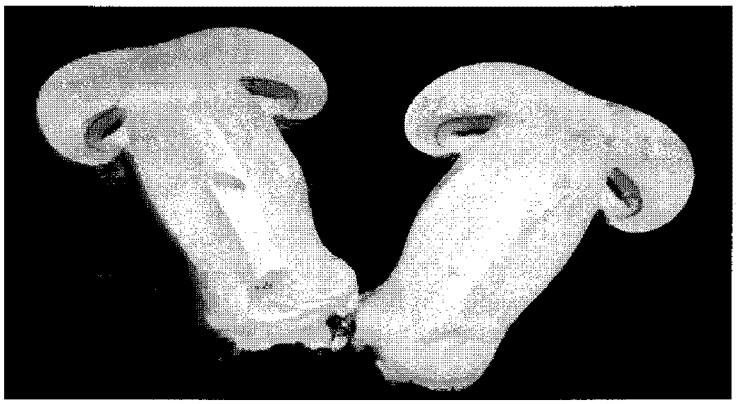


Fig. 10. Straight-cut fruitbody in manual cultivation of wild *A.bisporus* from Tibet.

2.2.5. The fruitbody gills were observed under microscope (400X), and it was found that the bisporus basidia occupy 70-80% and trisporus basidia 20-30% of the total basidia (Fig. 11).

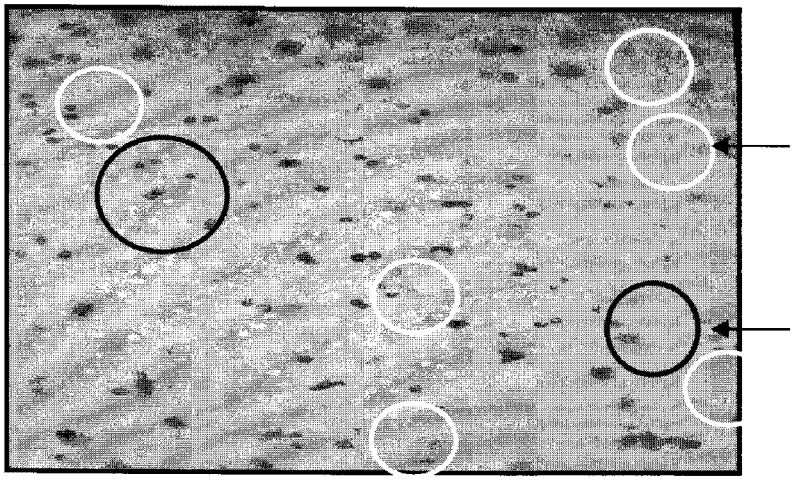


Fig. 11. Microscope observation (400X) of the basidia of wild *A.bisporus* from Tibet.

2.2.6. Monospores were separated from wild strain Ag4-3, and most of the monospore isolates inherited the parent growing character with appressed and weak mycelia, and fruited in cultivation. Only one isolate Ag43-1 grew slowly, and was identified as homokaryotic sterile isolate by isozyme electrophoresis. It could be hybridized compatibly with homokaryotic sterile monospore isolate ARP159-5 derived from ARP159, and produced the strain growing and fruiting normally.

3. DISCUSSION

In this study the two kinds of technologies, SRAP and ISSR, were used for analyzing Chinese wild *Agaricus* strains, and a dendrogram was obtained. Some strains had lower coefficient values, which might result from the primers selected and the bands counted. The statistical analysis based on more primers and amplified bands will result in more veridical similarity values, but have few effects on the clustering results. Most of the 44 *A.bisporus* strains identified preliminarily by isozyme electrophoresis were clustered as a big group at DNA level, suggesting the feasibility of *A.bisporus* identification by isozyme electrophoresis, and the mutual confirmation of isozyme and DNA markers in *A.bisporus* identification.

The wild strains produced less and easily opening fruitbodies, resulting in lower production. It could be regarded as a kind of adaptation of self-protection answering the bad environment, which should do benefit to the strains surviving. But their ability of low temperature and high temperature tolerance, was exactly the characteristics to be improved in commercial strains, and was greatly valuable in genetic breeding.

The isozyme patterns of the wild strains belonged to H type, indicating them to be traditional strains not crossed [13,14]. But their RAPD phenotypes were different from those of the strains introduced overseas, suggesting that the Chinese wild *A.bisporus* strains make some genetic difference from occident wild *A.bisporus* strains.

REFERENCE

[1] Wang ZS, 2000. Cultivation and strain improvement of *Agaricus bisporus* in China. Edible Fungi of China, 19(Suppl.): 33-36. (In Chinese).

[2] Li Y,1990. New species and records of the genus *Agaricus* from CHina[J]. *Acta Botanica Yunnanica*, 12(2):154~160 (In Chinese).

[3] Wang ZS, Liao JH and Li FG, 1993. Identification of field-collected isolates of *Agaricus bisporus*[J]. *Micologia Neotrpicla Aplicada*, (6): 127-137.

[4] Wang B, Tang LM and Li H, 2001. Identification and cultivation of wild *Agaricus bisporus*[J]. *Edible Fungi*, 23(Suppl.): 109-116.

[5] Wang ZS, Liao JH and Li HR et al., 2008. Study on the Biological Characteristics of Wild *Agaricus bisporus* Strains from China[J]. *Mushroom Science*, XVII: 149-158.

[6] Chen MY, Liao JH, Li HR et al., 2009. DNA fingerprinting of genetic diversity of *Agaricus bisporus* cultivation strains[J]. *Chinese Agricultural Science Bulletin*, 25(04): 149-156 (In Chinese).

[7] Li G, and Quiros CF, 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*[J], *Theor Appl Genet*, 103: 455-461.

[8] Zhang QL and Luo ZR, 2004. ISSR technology and its applications in fruit trees[J]. *Journal of Fruit Science*, 21(1): 54-58 (In Chinese).

[9] Wang ZS and Liao JH, 1990. Study on the crossbreeding techniques of *Agaricus bisporus*. Microl.Neotrop.Apl., (3): 1-12.

[10] Wang HC and Wang ZS, 1989. A study on screening isozyme markers of *Agaricus bisporus*. Edible Fungi of China, (6): 7-12. (In Chinese).

[11] Wang HC and Wang ZS, 1989. The prediction of strain characteristics of *Agaricus bisporus* by the application of isozyme electrophoresis. Mushroom Science, 12(1): 87-100.

[12] Chen MY, Liao JH and Wang ZS, 1998. Study on the RAPD amplification of three types of *Agaricus bisporus* strains. Acta Edulis Fungi, 5(4): 6-10. (In Chinese).

[13] Wang ZS and Wang HC,1989. Study on the genetic variation of *Agaricus bisporus*. Mushroom Biotechnology, Proceedings of the International Symposium on Mushroom Biotechnology(Nanjing, China), P: 329-338.

[14] Wang ZS and Wang HC, 1990. Isozyme patterns and charateristics of hybrid strains of *Agaricus bisporus*. Microl. Neotrop.Apl., (3): 19-29.