*Rept. of Toyo J. Coll. of Food Technol. & Toyo Inst. of Food Technol.*, 21, 11–14 (1996)

# Stability in Productivity of Protoclones from the Cultivated Mushroom, *Agaricus bisporus*

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Two protoplast regenerates (protoclones) PP-31 and PP-44 were selected by tray culture (Kasetani *et al.* 1992). After six-months storage, the two protoclones were cultivated again to examine stability of their viability and characteristics. Their yields and percentages of commercially valuable fruiting body ('button') were still higher than parental strain Horst U3$^R$ (control). At least, increase of yield of protoclones was maintained in six months. This result suggests a possibility which new valuable and stable strains will be obtained by protoplast production and regeneration.

Key words : mushroom, *Agaricus bisporus*, protoplast, protoplast regenerate, protoclone, storage, yield, button.

The mushroom *Agaricus bisporus* (J. Lange) Imbach is one of the most important cultivated mushrooms produced and consumed worldwide. Therefore, *A. bisporus* includes various strains in agronomic characteristics, for example, fruiting body's color and shape, cropping pattern and so on. However its effective and selective breeding is problematic, because of lacking clamp connection and secondary homothalism with few haploid propagules. Recently, protoplast production and regeneration and/or fusion (Maeta *et al.*[11], Royer *et al.*[12], Horgan *et al.*[5], Wang *et al.*[13], Jin *et al.*[6], Lodder *et al.*[10]), restriction fragment length polymorphisms (Castle *et al.*[1,2]), and other techniques in genetic engineering (Khush *et al.*[9]) were introduced to improve *A. bisporus* (Elliott[3], Wood[14], Kerrigan *et al.*[8]) strains.

We attempted to produce and regenerate protoplasts of *A. bisporus* as first step to efficient and selective breeding. In previous study, we obtained 60 protoplast regenerates, so-called PRs or protoclones, then examined them and selected two protoclones on the bases of radial growth ratio of mycelium, colony morphology, yield and characteristics of fruiting body in tray culture (Kasetani *et al.*[7]).

In the present study, we cultivated again the protoclones on tray after six-months storage on PDA slants, and examined productivity and quality of fruiting body.

## Materials and Methods

### 1. Materials

#### 1) Culture media and substrate

PDA medium was used for maintenance of *A. bisporus* strains. Wheat grain medium was used to prepare spawns for fruiting trial. Artificial compost and peat moss (casing soil) were used as substrates of fruiting trial. The preparation methods of these media were described previously.

2) Protoclones and control strain

Two protoclones PP-31 and PP-44 were produced from a commercial hybrid strain Horst U3$^R$ in the previous study (Kasetani *et al.*[7]), and maintained on PDA slants under 2℃.

After six-months storage, the two protoclones and control strain (Horst U3$^R$) were inoculated and incubated in 100g of wheat grain media filled in 300 mℓ-Erlenmeyer flasks as spawns.

2. Method of fruiting trial

Fruiting trial was carried out in growing room of this institute using plastic boxes (Sanbox #36-2M, Sanko Co., Gifu, Japan) as culture trays. 10 kg of the compost was filled in each box.

In past trial, all fruiting bodies grew as large as possible just before 'cup' to evaluate agronomic characteristics of each protoclone. In the present trial, most of fruiting bodies were picked at a commercial cropping stage 'button' and M size (28 to 35 mm) of pileus diameter. 'Button' means immature fruiting body which partial veil covering gill is still firm. 'Cup' means mature fruiting body which partial veil slightly tears and gill becomes visible. The cropped fruiting bodies were cut at their stipe-end, and classified based on their mature stage and size, then weighed and counted. The yield is expressed as $kg \cdot m^{-2} \cdot 3weeks^{-1}$. Except culture place, the growing condition of *A. bisporus* and criteria of fruiting body' classification were same as the previous study.

## Results and Discussion

In the previous study, we selected two protoclones PP-31 and PP-44, because of the highest button ratio and the highest yield. In this study, the two protoclones were cultivated in the growing room after six-months storage. Culture condition was improved on relative humidity (85% RH or more) and wind speed (less than 30 cm/sec) of climate in the growing room, comparing with those of the incubators used previously. Low wind speed kept in moisture of the substrates, particularly the casing soil. Alteration of cropping stage and climate of the growing room improved the fruiting bodies' quality and yield.

Fig. 1 shows yields in the fruiting trial. The two protoclones' button increased greatly, and were still higher than the control in whole and button yields and ratio of button. Particularly, button yield of PP-31 swelled and whole yield doubled. PP-44 was stable in the whole yield between previous and present trial, while button yield increased largely. Though the button yield of the control increased slightly, the whole yield decreased, because cup yield dropped.

High humidity and low wind speed of the growing room are suitable climate for *A. bisporus* cultivation. The climate and harvest at commercial cropping stage increased greatly the button yield, and decreased the cup yield. However, the strains' reaction to the climate was different. PP-31 was influenced heavily by the climate. Its yield was high in suitable climate of the growing room and low in bad climate of the incubator. PP-44 was rather stable in contrast to PP-31. Its whole yield did not change significantly. However, its button yield increased mainly by modifying of the cropping stage. It is suggested that PP-44

is less susceptible to dry, bad culture condition. The cause of dropping of the control's yield was not evident now.

Fig. 2 shows the classification of button fruiting body based on pileus diameter, which is expressed by percentages in the yields. Although only 20% of the button of the control was L size previously, L size fruiting bodies increased to approximately 40% in this trial. This increasing may be caused by relative low yield and improved climate. The protoclones indicated no significant change of the percentages in the sizes of fruiting bodies. Those are generally influenced by harvesting stage rather than the growing climate. In this case, harvest at commercial cropping stage was mostly M size. This cropping stage disguised the difference in size among the protoclones and the control.

## Conclusion

Even though we did not genetically

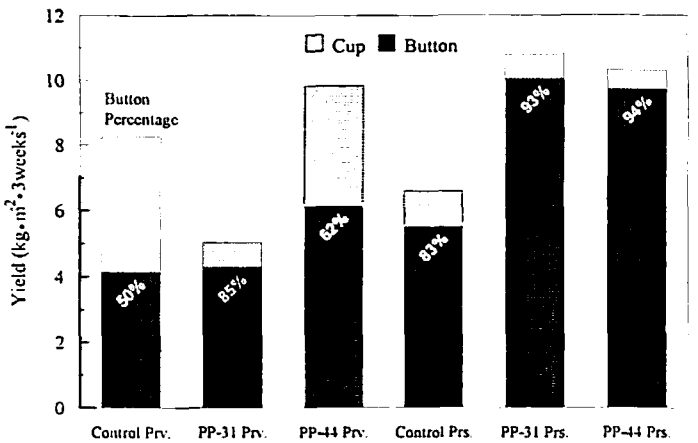


Fig. 1 Comparing of yields in previous and present fruiting trial.
Control is Horst U3$^R$, a commercial hybrid strain and the material of protoplasts.

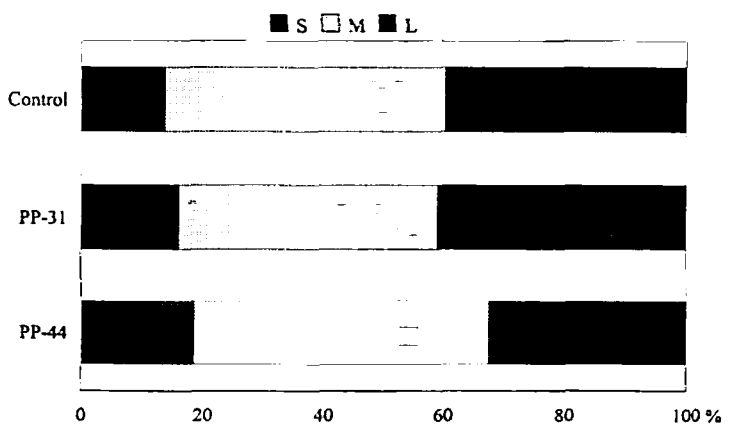


Fig. 2 Percentages of S, M and L sizes of button fruiting body.
Pileus diameter: S; 21-28 mm, M; 28-35 mm, L; 35 mm or more.

improve the protoplasts in the previous study, the protoclones' characteristics changed in radial growth ratio and colony morphology, yield and size of fruiting bodies. The cause of this change seems to us that the process of protoplast production and regeneration acts as a screening for vital and/or genetically varied cells concealed in the parental colony. We emphasize the former, screening of vital cells, because 3% (two in 60 protoclones) of the frequency is too high for genetic variation.

The two protoclones, PP-31 and PP-44, surpassed the control in yield and/or quality after six-months storage on slant medium. As a result of the two fruiting trials, they did not degenerate in their yields and quality. Therefore, the fertility of selected protoclones remained at least for six months. Fukumasa-Nakai also confirmed that the fertility of protoclones of the Shiitake, *Lentinula edodes* was stable during four-years subculturing on slant medium (Fukumasa-Nakai *et al.*[4]). However, further examination is necessary to confirm whether the fertility of *A. bisporus* protoclones is temporary or permanent. In this study, We demonstrate a farther possibility that strains of *A. bisporus* may be improved by protoplast production and regeneration with screening.

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