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Development of Straw Mushroom Strain for High Yield by Gamma Radiation

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Abstract The yield of straw mushroom was depended on each strain of straw mushroom. However, straw mushroom had genetic variation and rather high degeneration. Therefore, the improvement of straw mushroom strain by radiation for higher yield than the parent strain should be investigated. Mushroom mycelia were gamma irradiated at 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 kGy. Mutant colonies induced by radiation were totally selected 161 isolates. Among 161 isolates of suspected mutants, 153 isolates were fertile and 8 isolates were sterile. The preliminary test revealed that 153 isolates of fertile mutants, 59 isolates gave higher yield than the parent strain. For confirmation of the ability of screened mutants and the stability test, it was found that 13 isolates of total 59 isolates still showed higher yield than the parent strain and were still stable. Among 13 isolates of stable mutants, No.0.25-17 (irradiated at 0.25 kGy) was the best mutant. The experimental data of three screened mutants for high yield were evaluated for statistical analysis. It was found that there were significant differences in productivity among two isolates of mutants and the parent strain. However, only mutant strain (No.0.25-17) showed highly significant differences from the parent strain in both fruiting bodies and short cultivation period. Furthermore, three screened mutants and the parent strain were investigated for optimum culture conditions on temperatures, pH, formula of the media, carbon and nitrogen sources for mycelial growth. The optimum temperature for mycelial growth of three mutants and parent strain on potato dextrose agar were 37°C. According to the results, the mushroom complete medium was the best culture medium for mycelial growth of all isolates. The optimum pH for mycelial growth of parent strain and three mutants were 8.0, 8.5, 8.5 and 8.5, respectively. Mannitol was the best carbon source for mycelia growth of all isolates whereas lactose was the least. Nitrogen sources which supported the greatest growth of parent strain and three mutants were yeast extract, peptone, ammonium nitrate and ammonium nitrate, respectively. The statistical analysis of all factors affecting mycelial growth revealed that there were no significant differences in all factors tested except carbon sources.

Keywords: Gamma radiation, Straw mushroom, Mutant, Yield, Optimum culture condition

Introduction

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In Thailand, the production of straw mushroom was about 84,000 tons per year (Department of Agricultural Extension, 2000) which was the highest productivity in comparison with other kinds of mushrooms. According to the favourite in the good taste and high nutritive values of straw mushroom, the demand of consumers is more than the suppliers. Moreover, straw mushroom has the pharmaceutical properties such as healing function for hypertension, high blood cholesterol level, an auxiliary treatment for cancer and diabetes patients (Anonymous, 2011).

There are four strains of straw mushroom that generally used for cultivation. The strain used for cultivation in Thailand is *Volvariella volvacea* Fr. (Parnutat, 1997). The productivity of straw mushroom was depended on each strain of straw mushroom. However, straw mushroom has genetic variation and rather high degeneration (Petcharat, 1993). Therefore, development of straw mushroom strain by gamma irradiation for higher yield than parent strain should be investigated. Lastly, the new isolates that had higher yield would be kept in proper conditions to maintain the high efficiency so that genetic traits and the productivity of straw mushroom would be stable.

Materials and methods

Straw mushroom strains

Straw mushroom strain No.9 received from Department of Agriculture (Thailand) was used as a parent strain. Three screened mutant isolates (No. 0.25-17, No. 0.25-29 and No. 1.25-10) for high yield were used for study on optimum culture conditions on temperatures, pH, culture media, carbon and nitrogen sources for mycelial growth.

Preparation of samples for irradiation

Mushroom mycelia were cultured on Potato Dextrose Agar (PDA) at 30^{o}C for 7 days. The mycelia were scraped off the surface of the plates and put in the mixture solution of 0.01% Tween 20 and 0.85% NaCl. The suspended mycelia in the solution were homogenized for one minute 3 times in order to cut into tiny fragments. Afterwards, the tiny fragments were shaked well by vortex mixtures and transferred to 6 test tubes. Each test tube contained of 2.5 ml of suspended fragments was put in fitting aluminium rack for irradiation.

Gamma irradiation

A Co-60 irradiator (Gamma cell-220) at Office of Atoms for Peace, Thailand was used for this study. The dose rate determined with gamma chrome and radiochromic film dosimeters was 11.52 kilogray per hour (kGy/hr). The doses used for induction of mutation were 0, 0.5, 0.75, 1.0, 1.25 and 1.5 kGy.

Isolation of suspected mutant

The irradiated mycelia were diluted serially with the mixture of 0.01% Tween 20 and 0.85% NaCl. Each 0.1 ml of optimum dilution was spread on PDA plate and duplicate plates were done. All plates were incubated at 30^{o}C for 7 days to examine mycelial colony morphology. The suspected mutant colonies were sub-cultured on PDA slants as stock cultures.

Preparation of spawn

Exponential phase of mycelia of parent strain and the suspected mutants were cut into 2 cm diameter by cork borer and put on the surface of composted substrates for spawn making in plastic bags. The bags were incubated at 30^{o}C for 10 days or until the mycelia were full of the bags.

Preparation of substrates for cultivation

450 g of rice straw and 150 g of dried water hyacinth were soaked in tap water for 4 hrs. The water was drained out and the rice straw mixed with water hyacinth were exposed to the air overnight. After the moisture contents of the substrate were about 65-70%, the mixture was put in the plastic bags and kept overnight at room temperature. The mixture was mixed well with 10% commercial supplements and the final moisture contents were adjusted to be about 65-70%. The mixture was divided into 3 portions and packed each tightly to be round shape in plastic bags. The substrate bags were disinfected by autoclave.

Sowing

Each plastic bag was sowed with 16 g of spawn on the disinfected substrate. The plastic bags were tied up loosely with rubber bands and put them in a dark chamber (32-38^{o}C, little ventilation) for 2-3 days or until the mycelia covered on the surface of substrate.

Management for bottoning period

Management for bottoning period was carried out according to Jun-Cao Technology (Anonymous, 1998).

Harvesting of straw mushroom

The buttons came out after sowing for 9-12 days. For harvesting, one hand was put on the rice straw around the button of the straw mushroom, then the other hand was used to pick it up by swirling lightly. The second flush mushroom would appear in 5-6 days. In this experiment, the whole period for cultivation of straw mushroom from sowing spawn to harvesting (2-3 times) was about 25 days.

Stability test

The screened mutants for high yield were incubated at 30^{o}C or room temperature for 3 months. The mutants were sub-cultured on fresh PDA medium for 5 times. The abilities of screened mutants were tested for higher yield than the parent strain in each time of subculture.

Biological efficiency was calculated on the basis of a ratio of wet weight of basidiocarps (Chang *et al*., 1981).

$$\text{B. E. (\%)} = \frac{\text{Wet weight of basidiocarp}}{\text{Dry weight of substrate}} \times 100$$

Effect of Temperatures on Mycelial Growth of Parent and Mutant Isolates

Exponential phase of mycelia of each isolate were cut into 10 mm discs. Each disc was put on the center of potato dextrose agar (PDA) plate, pH 6.8. Then the plates were incubated at 25℃, 30℃, 35℃, 37℃ and 40℃ for 3-7 days. Duplicate plates were done in each temperature. After incubation, the diameter of mycelia were measured everyday until each plate was full of mycelia.

Effect of pH on Mycelial Growth of Parent and Mutant Isolates

Mycelial disc (10 mm) of each isolate was put on the center of PDA plate which varied pH from 5.5 to 9.0. Duplicate plates were done in each pH and all plates were incubated at 30℃ for 3-5 days. After incubation, the diameter of mycelia were measured everyday.

Effect of Culture Media on Mycelial Growth of Parent and Mutant Isolates

Mycelia disc (10 mm) of each isolate was put on the center of each culture medium plate.The culture media used in this study were mushroom complete medium (MCM), potato dextrose agar (PDA), malt extract-yeast extract agar (MYA) and potato dextrose straw extract agar (PDSA). Duplicate plates were done in each culture medium and all plates were incubated at 30℃ for 3-5 days. After incubation, the diameter of mycelia were measured everyday.

Effect of Carbon Sources on Mycelial Growth of Parent and Mutant Isolates

The control medium was mushroom minimal medium without carbon source. Various kinds of carbon sources tested were sucrose, mannitol, glucose, fructose, lactose, xylose and arabinose. The concentration of each carbon source was 2%. Mycelial discs (10 mm) of all isolates were put on the center of the plates containing mushroom minimal medium with different carbon sources. Duplicate plates were done in each carbon source and all plates were incubated at 30℃ for 3-11 days. After incubation, the diameter of mycelia were measured everyday.

Effect of Nitrogen Sources on Mycelial Growth of Parent and Mutant Isolates

The control medium was mushroom minimal medium without nitrogen source. Seven nitrogen sources were ammonium chloride, peptone, ammonium nitrate, yeast extract, potassium nitrate, urea and L-asparagine. The concentration of each nitrogen source was 0.2.% Mycelial discs (10 mm) of all isolates were put on the center of mushroom minimal medium plates which varied nitrogen sources. Duplicate plates were done in each nitrogen source and all plates were incubated at 30℃ for 3-4 days. After incubation, the diameter of mycelia were measured everyday.

Statistical analysis

The experiment data from 3 replications were analyzed by using the SPSS software package. An analysis of variance and Duncan's multiple range test ($p < 0.05$) were applied to this study.

Results and discussions

As shown in Fig.1, the mycelial colony morphology of screened mutants were mainly selected the different phenotypic characteristics from the parent strain. The color of parent mycelia was violet brown whereas the color of screened mutants were rather white (light violet brown). For the types of hyphae, the parent was flat type and the mutants were rather aerial type. Jinxia and Chang (1992) reported that the mycelial colony morphology might be primary characteristics for identification of productivity and quality of *Volvariella volvacea*

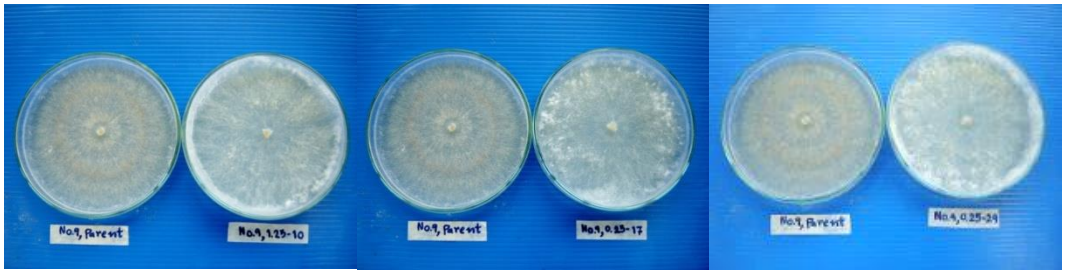

Fig. 1. Comparative mycelial colony morphology of parent strain and mutant isolates on potato dextrose agar. Plates on the left side in each picture were parent strain, on the right side were mutant strains induced by gamma ray

Table 1 shows screening of mutant isolates for higher yield than the parent strain. Screening of mutant isolates for higher yield than the parent strain is shown in Table 1. Mutant colonies induced by radiation were totally selected 161 isolates. Among 161 isolates, 153 isolates were fertile and 8 isolates were sterile. If the chlamydospores (red brown color) on the mycelia of spawn were found, it showed that the mutants were fertile as shown in Fig. 2. The preliminary test revealed that 59 out of 153 isolates of fertile mutants gave higher yield than the parent strain. For confirmation of the ability of screened mutants and the stability test, it was found that 13 isolates of total 59 isolates still showed higher yield than the parent strain as shown in Fig. 3 and were still stable. Among 13 isolates of stable mutants, the mutant No. 0.25-29 (irradiated at 0.25 kGy) gave the highest productivity (Fig. 4).

Table 1. Screening of mutant isolates for higher yield than the parent strain

Suspected mutants	Fertile mutants	Sterile mutants	Preliminary screening:higher yield mutants	Confirmative screening:higher yield mutants	Stability test
161	153 (95.03%)	8 (4.97%)	59 (38.5%)	13 (22.03%)	13 (22.03%)

Fig. 2. Mycelial growth and chlamydospores on substrates for spawn making

Fig. 3. Comparative productivities of parent and mutant isolates

As shown in Fig. 4, the biological efficiency (B.E.) of mutant No. 0.25-29, No. 0.25-17 and No. 1.25-10 were 42.94%, 42.23% and 36.25%, respectively whereas the B.E. of the parent strain was 30.28%. However, there were significant differences of productivity among two isolates of mutants (No. 0.25-17, No. 0.25-29) and the parent strain. Similar findings were also reported (Djajanegara, I., and Harsoyo, 2009) that mutant strain of oyster mushroom (irradiated at 0.75 kGy) showed significantly higher productivity compared to control.

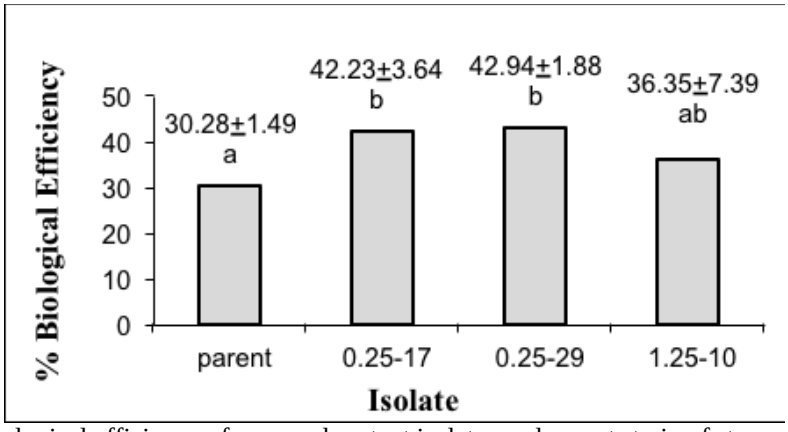

Fig. 4. Biological efficiency of screened mutant isolates and parent strain of straw mushroom

Fig. 5 shows number of fruiting bodies of screened mutant isolates and parent strain of straw mushroom. All mutants produced more fruiting bodies than the parent strain. On the other hand, the mutant No. 0.25-17 showed highly significant fruiting bodies from the parent strain which was in accordance with the data of Djajanegara and Harsoyo (2009).

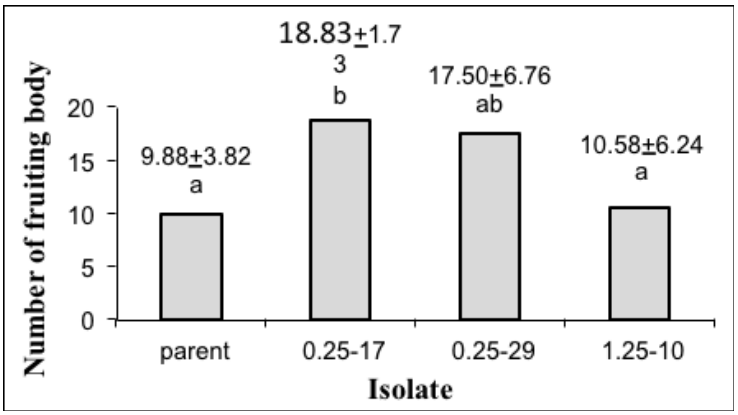

Fig. 5. Number of fruiting bodies of screened mutant isolates and parent strain of straw mushroom

Cultivation period of screened mutant isolates and parent strain of straw mushroom is shown in Fig. 6. All mutants took shorter cultivation period (9.63-10.50 days) than the parent strain (11.25 days). Based on statistical analysis, only the mutant No. 0.25-17 took significantly shorter cultivation period than the parent strain. Moreover, the results of this experiment made benefit for farmers to harvest straw mushroom earlier.

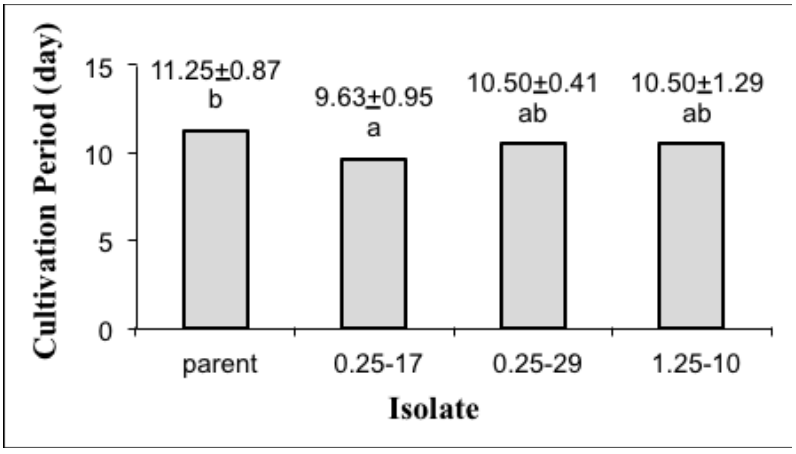

Fig. 6. Cultivation period of screened mutant isolates and parent strain of straw mushroom
Cultivation period : Sowing spawn to harvesting the first flush mushroom

Although mutant No.0.25-17 did not give the highest productivities, it was recommended as the best mutant. Based on our results, this mutant showed significant differences of B.E., number of fruiting bodies and short cultivation period from the parent strain whereas mutant No. 0.25-29 showed significant differences from the parent strain only in B.E. Moreover, both mutants were most likely to exhibit the B.E. values.

The effect of temperatures on mycelial growth rate of parent and mutant isolates is shown in Fig. 7. All isolates grew actively at 35-37℃, moderately at 30℃ and 40℃ and poorly at 25℃. The optimum temperature for mycelial growth rate of all isolates was 37 ℃. Kurtzman, and Chang-Ho (1982) reported that the optimum growth temperature of *Volvariella* mushroom was 35℃. In other literature search, the optimum temperature for mycelia growth was depended on strain of straw mushroom (Pukahuta, 1986). However, there were no significant differences for mycelia growth rates at all temperatures of each isolate. In addition, there were no significant differences for mycelia growth rates of all isolates at the same temperature.

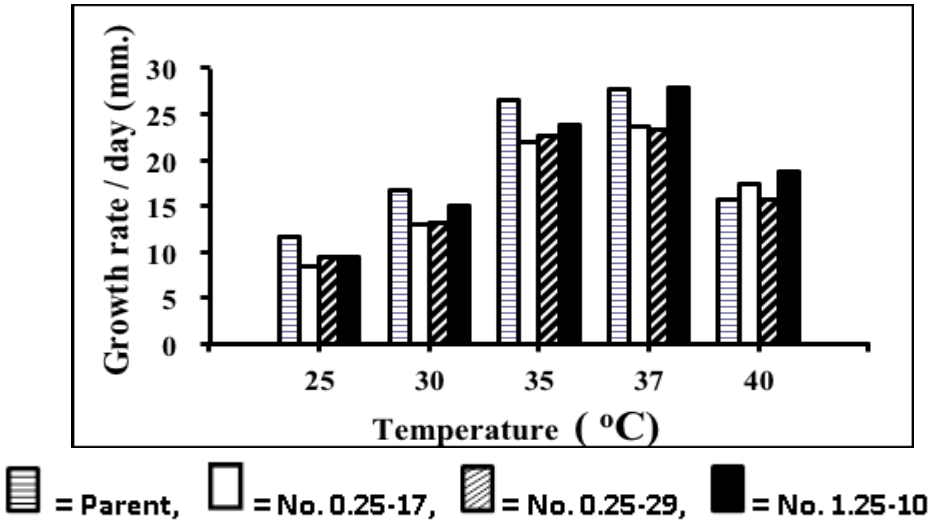

Fig. 7. Effect of temperatures on mycelial growth rate of parent and mutant isolates

The effect of pH on mycelial growth rate of parent and mutant isolates was investigated, as shown in Fig. 8. Based on our experiments, glucose was used as carbon source in culture medium (PDA) and the optimum pH for growth of parent strain and three mutants were 8.0, 8.5, 8.5 and 8.5, respectively. These results were in accordance with Tzeng (1974) indicated that the optimum pH for growth was 5, 8 and 9 when maltose, glucose and

pectin was used as carbon source, respectively. On the other hand, there were no significant differences for mycelial growth rates at all pH of each isolate. Furthermore, there were no significant differences for mycelial growth rates of all isolates at the same pH.

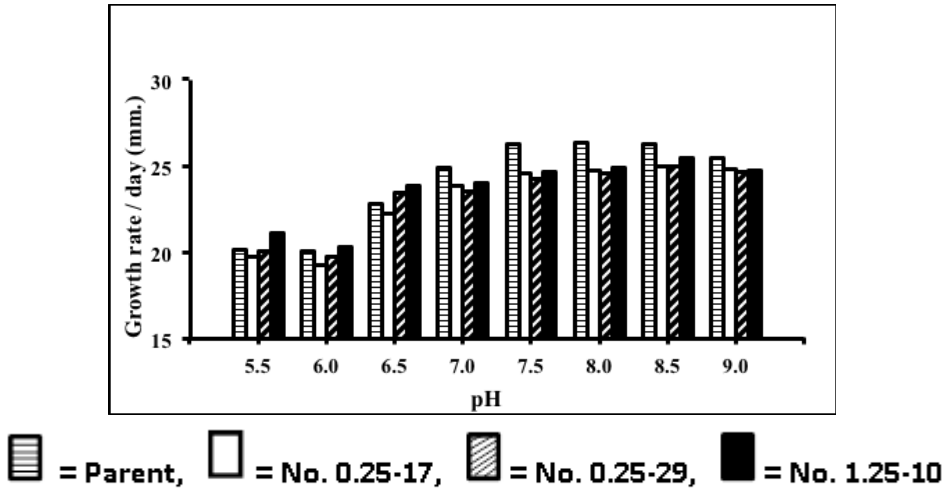

Fig. 8. Effect of pH on mycelial growth rate of parent and mutant isolates

As shown in Fig. 9, among the different culture media tested, the best mycelial growth rate of all isolates was on mushroom complete medium followed by malt extract-yeast extract agar followed by potato dextrose agar. The least mycelial growth rate of all isolates were on potato dextrose straw extract agar. On the contrary, Pukahuta (1986) reported that potato dextrose agar was the best culture medium for mycelial growth rates of three straw mushroom isolates and the worst culture medium for mycelia growth rates was malt extract-yeast extract agar. Although these results were not correlated to Pukahuta's experiment, the optimum culture medium for mycelia growth might be based on the strain of straw mushroom. For statistical analysis, there were no significant differences for mycelial growth rates of all isolates on the same culture medium. Moreover, there were no significant differences for mycelia growth rates on all culture media of each isolate.

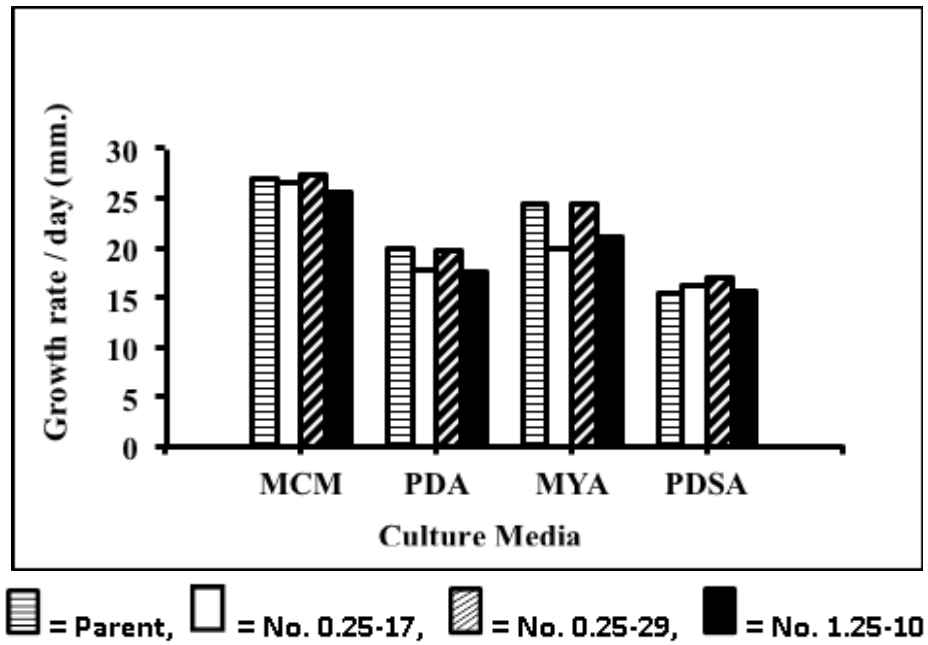

Fig. 9. Effect of culture media on mycelial growth rate of parent and mutant isolates; MCM : mushroom complete medium; PDA : potato dextrose agar; MYA : malt extract-yeast extract agar; PDSA : potato dextrose straw extract agar

The effect of carbon source on the mycelial growth rate of parent and mutant isolates was shown in Fig. 10. Mannitol supported the greatest mycelial growth rates of all isolates as a carbon source. Growth on sucrose was nearly equal to growth with mannitol which is correlated to result of Pukahuta (1986). However, lactose supported the least mycelial growth rates of all isolates.

Similar results were obtained by Chandra and Purkayastha (1977) that lactose supported little or no growth. Based on statistical analysis, there were significant differences for mycelia growth rates among lactose and other carbon sources of each isolate. However, there were no significant differences for mycelial growth rates of all isolates by using the same carbon source.

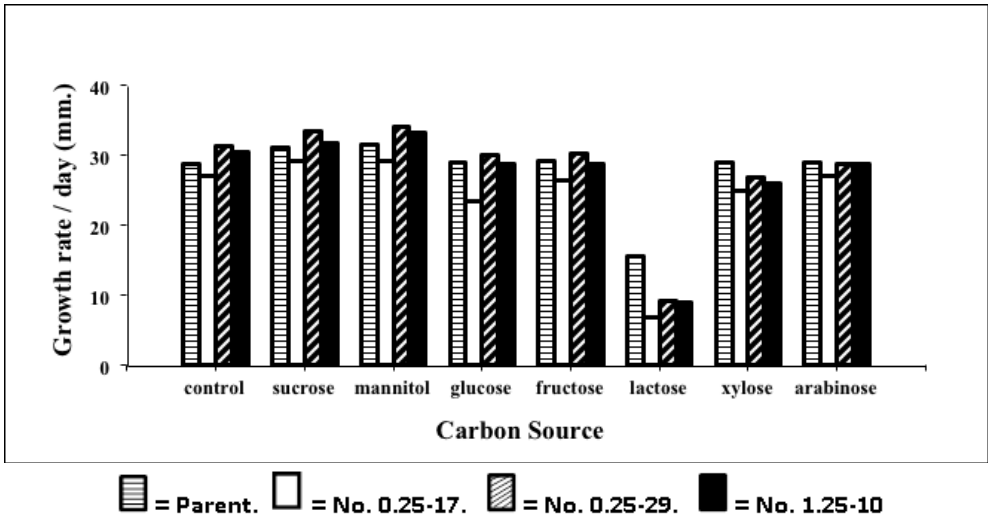

Fig. 10. Effect of carbon sources on mycelial growth rate of parent and mutant isolates

As shown in Fig. 11, the results revealed that the utilization of nitrogen sources were varied by each isolate and the kind of nitrogen source. In our investigation, the parent strain grew best on yeast extract which was in accordance with the data of Tzeng (1974). However, mutant No. 0.25-17 grew best on peptone. Similar results of Chandra and Purkayastha (1977) and Voltz (1972) also indicated that the greatest growth was supported by peptone. On the other hand, ammonium nitrate supported the greatest growth of both mutant No. 0.25-29 and No. 1.25-10 which was correlated to the results of Pukahuta (1986). In contrast, urea was toxic (Tzeng, 1974) and supported only about ten percent of the growth supported by asparagine (Garcha *et al*.1979). Thus, urea supported the least growth of all isolates in our experiment.

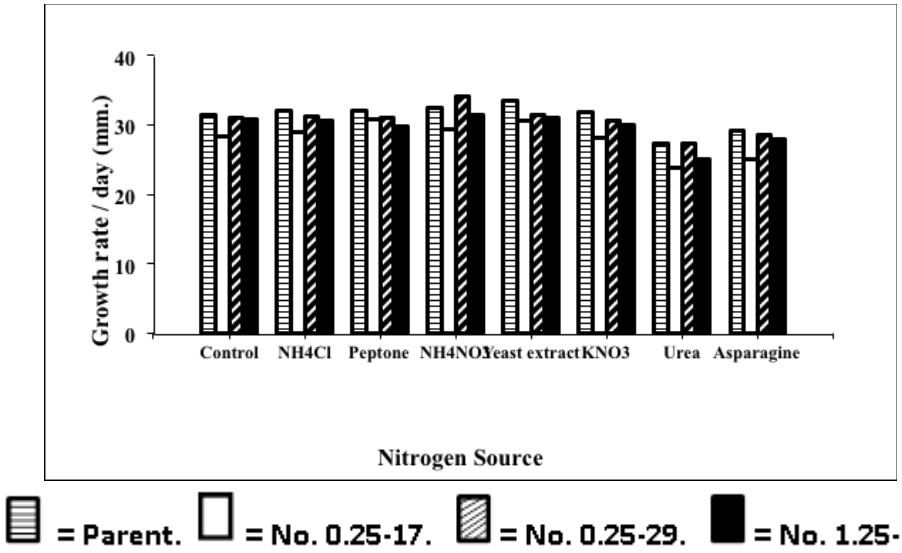

Fig. 11. Effect of nitrogen sources on mycelial growth rate of parent and mutant isolates

Conclusion

Thirteen mutant isolates induced by gamma radiation were selected for higher yield. Three screened mutants for high yield were evaluated for statistical data analysis. It was found that there were significant differences in productivity among two isolates of mutants and the parent strain. The isolate No.0.25-17 (irradiated at 0.25 kGy) was screened as the best mutant. Furthermore, three screened mutants and the parent strain were investigated for optimum culture conditions on temperatures, pH, formula of the culture media, carbon and nitrogen sources for mycelial growth. The statistical analysis of all factors affecting mycelial growth revealed that there were no significant differences of all factors tested except carbon sources. Therefore, gamma radiation could be used for development of straw mushroom strain for significantly higher yield.

Acknowledgements

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