Laboratorial Steady Culture Conditions of a Mushroom for Assaying Growth Factors

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Summary

Brief culture methods of a fungi (*Pleurotus ostreatus*) were tried to be introduced for the assay of like growth factors. Diameter of bottles and quantity of solid medium were tested for this purpose under the fixed conditions of both humidity and radiation of light. From the results, the bottle of 32mm outside diameter and 15g solid medium was accepted as suitable conditions. These conditions reduced culture period, being 24 days, and moreover, made possible one step culture of mushroom for the experiment of the above assay.

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Key words : *Pleurotus ostreatus*, growth-factor assay, mushroom culture method.

Introduction

There have been many reports on culture of mushrooms for searching biologically active substances like growth factors (Hashimoto and Takahashi,[1] ; Teranishi et al.,[2] ; Yoshikawa and Tsuetaki,[3] ; Teranishi et al.,[4] ; Murao et al.,[5] ; Hayashi et al.,[6] ; Hayashi et al.,[7] ; Azuma et al.,[8,9]). In such case, agar media are generally used as a brief method for their culture. When such search made a success, a subsequent experiment should be generally achieved on a medium which was commercially used for mushroom cultivation to put such substance to practical use. Furthermore, uniformity of growth of fruitbody in each culture flask should be kept as constant as possible, otherwise it results in an undesired increase in the number of sample set in a culture batch. A method completing the experimental culture for the above assay by one step, if any, will save time, and the number of sample set in a culture batch will be reduced effectively. From this point of view we proved such method by which mushroom culture is able to be achieved easier.

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Materials and Methods

Test Fungus *Pleurotus ostreatus* (Jacq.: Fr.) Kummer was chosen as a delegate for the present culture method, because it is commonly cultivated in Japan.

Medium The saw dust was washed with water and dried up at 70°C for 18h after autoclaving at 120°C for 20min. It was blended with the wheat bran in a ratio of 4 to 7 (v/v). After then water was added into the blender up to 65 % of the total weight. The medium so prepared was bottled and referred to as the present culture medium

Culture The bottles of 23, 32, and 40mm outside diameter ("diameter" in the following sentences means outside diameter) and 100mm high each were used for the culture. The medium prepared above was packed into the bottles with set quantity of 5, 10, 15, 25, and 35g. The culture bottles were settled in an incubator (Nippon Medical & Chemical Instruments Co. Ltd., LPH-200-SD) controlled at 25°C and ca. 70 % humidity in dark, after inoculating the mycelium. When the mycelium was grown to the bottom of the medium in more than a half bottle of one experimental batch, an excess of mycelium grown on the medium surface was removed off. The bottles were then filled up with tap water and settled for 20min. After then excess of the water was removed. A subsequent culture to gain fruitbodies was achieved in the incubator which was already controlled at 13°C and 85 % humidity at 100 lux irradiation.

Sampling The growing fruitbodies were harvested at the time when the ones growing slowest in a experimental batch became 10mm long. They were cut off at the

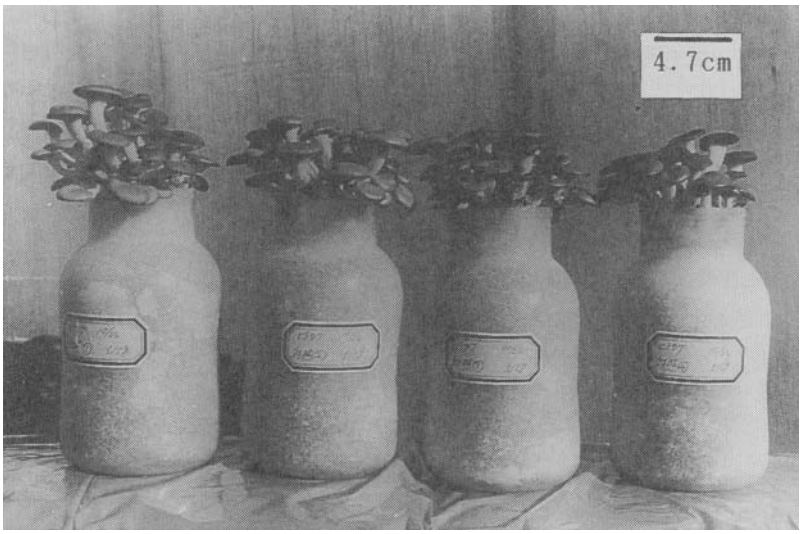

Fig. 1. Culture with 850 ml polypropylene bottle.

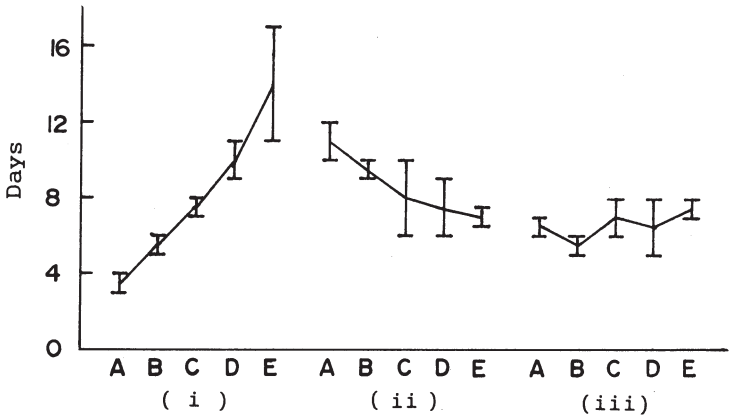

Fig. 2. Period required for each culture stage by 32mm bottle
(i) : mycelial growth, (ii) : primordium formation,
(iii) : fruitbody harvest. Culture medium; A : 5g, B : 10g, C : 15g, D : 25g, E : 35g
Verticals show probable errors.

bottom of stem and dried up in a desiccator at 70°C for 18h. The dried sample was weighed.

Results

Fig. 1 shows the fruitbodies produced by the culture with polypropylene bottles which are commercially used (850ml). The mature fruitbodies in this case were harvested in about 30 days after the mycelium inoculation. Fig. 2—5, on the contrary, show the results by the bottles used in this work. The experiments were carried out by measuring

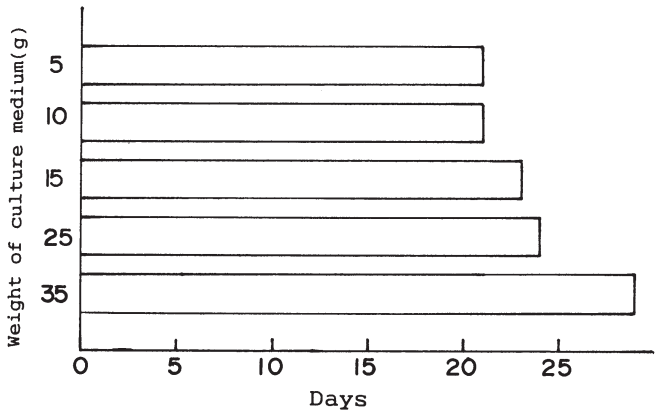

Fig. 3. Period required for harvest of fruitbody.

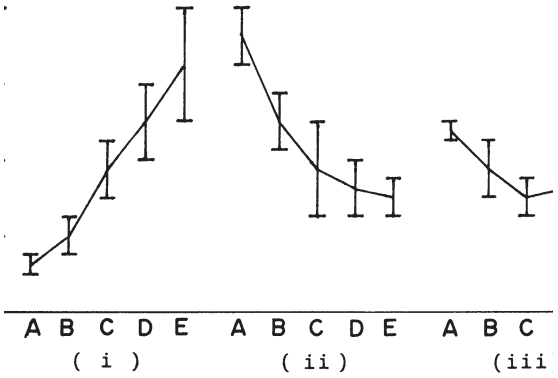

Fig. 4. Period required for each culture stage by 40mm bottle
Notations are the same as in Fig. 2. Verticals show probable errors.

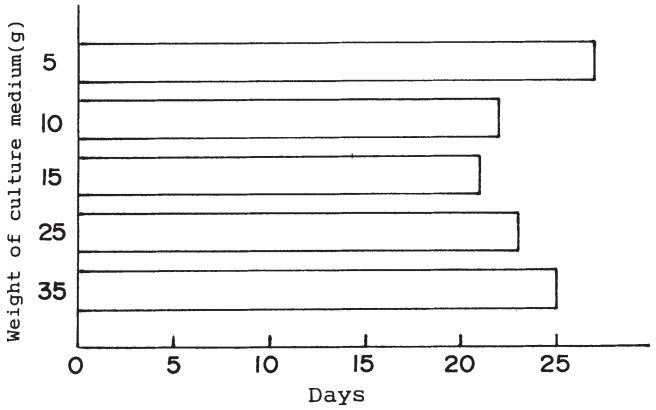

Fig. 5. Period required for harvest of fruitbody.

Table 1. Yield and number of fruitbody. Yield is shown in dry weight.

Diameter(mm)	Medium(g)	Yield(mg)	Number
40	5	49±11	1.3±0.2
	10	65±6	1.8±0.3
	15	172±29	3.7±1.1
	25	235±39	6.0±1.3
	35	347±87	7.4±1.6
32	5	36±5	1.5±0.2
	10	53±4	2.8±0.2
	15	173±22	3.2±0.4
	25	235±30	4.4±0.5
	35	411±24	5.5±0.3

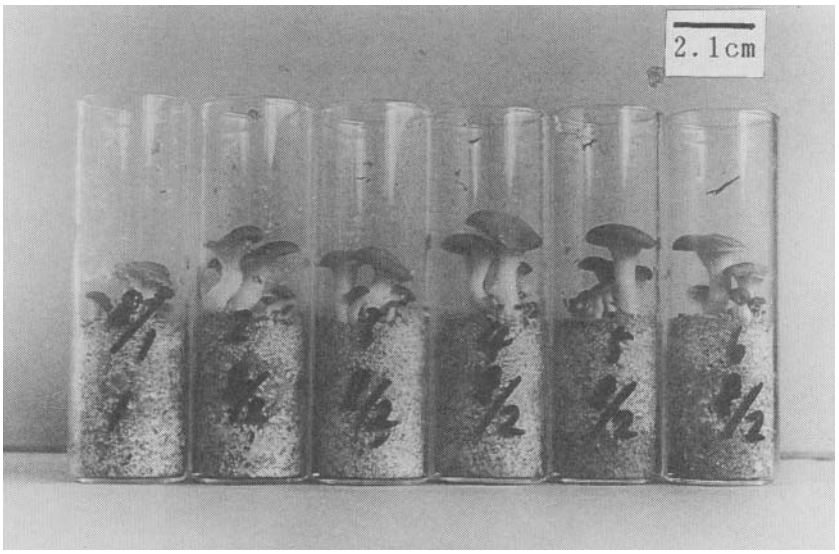

Fig. 6. Culture with 32mm−15g bottle.

the periods required for the following three stages ; (i) period for which the mycelium grows out in the medium, (ii) period for which the primordium starts to be formed on the mycelium, and (iii) period for which the fruitbody that is growing slowest of all the fruitbodies in each bottle becomes 10mm long. The above experiments were carried out on different quantities of the medium of 5, 10, 15, 25, and 35g. Fig. 2 shows the results with 32mm diameter bottle in which the period (i) was substantially prolonged with increasing the medium quantity. The period (ii), on the contrary, was lowered with increasing medium quantity. The period (iii), however, was given little variation against the change of medium quantity. Fig. 3 shows the total culture period by the bottle of 32mm diameter. In the case of the bottle of 40mm diameter, as shown in Fig. 4, the period (i) and (ii) have similar profiles of growth change with that of 32mm diameter bottle. The period (iii), however, showed a considerably different profile of growth change from that of 32mm bottle. Fig. 5 shows the total culture period by the bottles of 40mm diameter. The shortest total culture period for the experiments was 21 days and it was obtained in three cases : two cases of 5g and 10g medium by 32mm bottle (Fig. 3) and one case of 15g medium by 40mm bottle (Fig. 5). These cases, however, were omitted, because both (32mm-5g) and (32mm-10g) cases caused a large uneven aspect of the growth, that is the growth occurred in the space under the medium surface, due to dryness of the medium during the culture and because the (40mm-15g) case did large unevenness in the number of fruitbody, respectively. Table 1 shows the yield and the number of fruitbody in each conditions used above. These results demonstrate the less advantage in the case of the bottle 40mm diameter. Because the error of both yield and number of fruitbody is larger in the case of 40mm diameter bottle than 32mm diameter bottle. Taking up only fruitbody number

in both bottle size, the former is less suitable for the assay due to more number of fruitbody than the latter, because fruitbody in much number jostles in the bottle space to disturb its growth mutually.

Judging from the above results, the (32mm-15g) case was adopted to be the most suitable culture conditions for present purpose. The corresponding culture just before harvest is shown in Fig.6. We also tested by using 20mm outside diameter bottle, however, its result was removed completely here because of larger unevenness of data than those of other cases.

Discussion

There are many reports on investigation of mushroom growth-factors. In those reports, it is not described clearly how many sample set in one batch of experiment should be suitable for completion of investigation, while it will be undoubtedly obvious that the more the sample set in one batch is used, the stabler the data are obtained. An experimental equipment in a laboratory, however, usually has restriction, not permitting to stuff so many bottles in an incubator. We tried to make suitable experimental conditions such as bottle size and medium quantity under a limitted number of sample set of ten bottles.

This experiment was carried out by means of the fixed conditions of both humidity and radiation of light. If other conditions than these two are applied, different suitable culture conditions may appear. However, because the used conditions here should be normally accepted in many research laboratories, this method will be generally complied.

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きのこの成長因子などの探索に適用するための実験室的きのこ安定培養法

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きのこの成長因子などの探索を実験室的に行う際，利用できる恒温槽の大きさの制限から，１バッチ当りの試料本数と試料ビンの大きさは，できるかぎり小さいことが望ましい。さらに，通常，寒天培地で可能性のある物質のスクリーングをしたのち，実用的な培地でその効果を再度チェックする方法が採用されているが，その二重手間を省くことができれば，好ましいことは言うまでもない。著者らは，その達成を目的として，*Pleurotus ostreatus* をモデルとして培養実験を行った。その結果，外径32mm の培養ビンに，15g の固形培地を詰めた場合が，全培養期間を24日という短期間にすることを見いだした。しかもこの場合，一段階で実験を終了することができた。

キーワード：ひらたけ，成長因子検索，きのこ培養法。