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A Comparative Study on the Growth Rate of Homokaryotic Mycelium Obtained from a Single Spore of *Hericium erinaceus* Isolates in Different Culture Media

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The vast majority of research articles on physiology of macrofungi have used heterokaryons obtained from fruit body tissues. Actually there is evidence that heterokaryons and homokaryons exhibit differences in performances such as mycelium growth rate and properties like colony morphology etc. Ten homokaryons were obtained from a single spore germinated in 2% Malt Extract Agar medium, and their hyphal extension rates on %2 Malt Extract, Potato Dextrose, Hagem and Minimal Agar media were measured. Some significant differences were found in the mycelium growth rates of commercial *Hericium erinaceus*. Mycelium growth of only one homokaryon could reach to 90 mm diameter on the 27th days of incubation. Nine heterokaryotic mycelia formed at the pairing test performed on Potato Dextrose Agar.

Key words: *Hericium erinaceus*, Homokaryotic mycelium, Growth rate, Single spore isolate.

Compatibility of the mates of a mushroom occurs only when they differ in alles at both of the incompatibility loci as A and B mating types. Because of the sexual life cycle, both of homokaryotic and heterokaryotic stages are observed in these organisms. Depending on the mating types homokaryon-homokaryon interactions can be partially or fully compatible. In this bifactorial mating system the existence of homokaryotic or heterokaryotic mycelium is controlled by germinated spores, which were were also important to obtain individual homokaryon for use in breeding studies under lab conditions[1].

Species in *Hericium* genus (Aphyllophorales, Homobasidimycetes) are white and fleshy fungi, growing mostly on dead or dying wood. All of these species produce basidiomata as a mass of fragile icicle like spines that are suspended from either a branched supporting framework or from a tough, unbranched cushion of tissue; their basidiomata are considered as edible and delicious mushroom[2]. *Hericium* has attracted considerable attention for its various bioactive substances, and has been used in East Asia as an edible and medicinal mushroom to treat various human illnesses and diseases[3].

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As the pairing of a homokaryotic mycelium having different alles, almost completes in a heterokaryotic mycelium, this process creates discrete individuals. These are very important new hybrid strains for genetically research and spawn industries. However, the agronomical performance of new hybrid strains should be determined by using various microbiological methods and a cultivation test in a growing room. The intent of the present investigation was to isolate homokaryotic mycelium from germinated *Hericium erinaceus* spores and determine the mycelium growth rate on the different microbiological media described below. The crosses between homokaryotic mycelia were also investigated on Petri dishes.

MATERIAL AND METHODS

Collection and germination of basidiospores

The basidiocarps obtained from the mushrooms that were grown in growing room, were harvested and immediately transported to the laboratory. Whole basidiocarps were placed vertically in sterile Petri dishes and were left overnight for collection of their spores, and then 5ml of sterile water was added under aseptic conditions. The Petri dishes containing 5ml of water were gently shaken to collect most of the spores. The basidiospore suspensions were then transferred to sterile test tubes for preparation of a spore suspension. 1 ml of aliquots taken from the basidiospore suspensions was transferred to the sterile test tubes containing 9 ml of sterile water (1:10). Serial dilutions were performed until a 1:10000 dilution was reached[4].

One milliliter of each spore suspension was transferred onto the Malt Extract Agar (MEA) medium using sterile pipettes under aseptic conditions. Five replicates were prepared and all of them were incubated at 25±1ºC for 10 days. Vigorously germinating basidiospores, which were visible to the naked eye, were detected in front of the fluorescent lamp and were transferred onto the MEA.

Measurement of mycelial growth

Mycelial plugs (diameter 6 mm) cut under sterile conditions from the outer edge of the actively growing colony and all of the homokaryons and heterokaryons were transferred onto the centre of the Petri dishes containing Malt Extract, Potato Dextrose (PDA), Hagem (Malt extract 4.0 g, Yeast extract 1.0 g, D-glucose 5.0 g, NH_4Cl 0.50 g, KH_2PO_4 0.50 g, $MgSO_4$. $7H_2O$ 0.50 g, %1 $FeCl_3$ 0.50 ml, 100 ppm thiamin 0.125 ml, distilled water 1000 ml. pH 5.40-5.50) and Minimal (Urea 0.036 g, KH_2PO_4 2.0 g, $MgSO_4$.$7H_2O$ 0.50 g, $CaCl_2$ 0.099 g, D-Glucose 1.0 g, distilled water 1000 ml. pH 5.20-5.40) in Agar Media[5]. The plates containing the MEA were incubated at 24±1ºC for at least 30 days. The mycelium growth was followed by measuring the radial extension of the mycelium with a caliper gauge along two diameters at right angles to one another and then the average diameter for each plate calculated[6]. The mean mycelium growth was then calculated from the five replicates of each treatment.

Distinguishing *homokaryons* and heterokaryons by pairing test

Single basidiospore isolates were paired up with sibling and non-sibling combinations in Petri dishes. Ten putative homokaryons were crossed in all possible combination by pairing up with the PDA medium in order to produce the heterokaryons. Isolates were inoculated 3 cm apart from each other on the same Petri dishes. The heterokaryons were isolated from the interaction zone of both homokaryons. The heterokaryon cultures were then examined through the plates directly for evidence of the presence of clamps on the mycelium. Each cross was scored as positive (clamp formation) or negative (clamps not formed)[1].

Statistical analysis

The data presented are the average of the results of five replicates with a standard error of less than 5%.

RESULTS AND DISCUSSION

Spores germinated faster on the PDA, but contamination was high due to the rich content of this medium, so MEA or Minimal Agar was the preferred medium for the germination experiments. No special formulations were necessary to induce the germination of spores under the laboratory conditions. While spores were germinated on the MEA and PDA media in six days, they could do so in ten days on Minimal Agar.

After the microscopically examination, the selected ten homokaryotic mycelia were

transferred to various microbiological media to determine the mycelium growth rate. As shown in Table 1 and Figure 1, homokaryotic mycelia were exhibited at a different extension rate on the Petri dishes.

Strain 10, which was incubated in Hagem Medium and the MEA media, reached to a 90 mm diameter (coverage of agar plate) on the 27th day of incubation. On the other hand, strain 4 interestingly showed very slow growth patterns on all of the

Table 1. Mycelial growth rate of organisms (1 – 12) at 7th, 14th and 23rd days of incubation (mm)

	Incubation Days											
	7th				14th				23rd			
	PDA	HGM	MEA	MIN	PDA	HGM	MEA	MIN	PDA	HGM	MEA	MIN
1	7	16	8	7	14	35	15	24	23	50	21	34
2	7	10	8	7	11	23	17	18	19	27	20	20
3	15	19	12	7	34	34	29	24	45	45	43	32
4	7	7	6	6	10	11	7	6	15	28	25	6
5	8	10	10	11	17	25	16	20	21	32	22	26
6	9	11	21	8	17	23	39	21	34	42	58	29
7	13	18	8	8	23	33	20	16	31	40	30	21
8	15	18	10	6	25	55	32	7	33	72	50	12
9	9	16	10	6	20	38	24	8	38	54	39	13
10	28	24	29	11	56	52	64	17	77	88	90	44
11*	14	10	28	6	39	31	62	8	65	66	90	10
12*	8	10	10	7	11	34	35	18	27	64	70	48

*: heterokaryotic mycelium; HGM: Hagem medium; MIN: Minimal medium

media used in this study, with the single exception of the Minimal Agar Medium,. Although similar results were obtained with strain 5 and strain 2 at the beginning of the experiment, they showed a small amount of growth on the microbiological media on the 23rd and 27th day, and no mycelium growth at strain 4 was observed on the Minimal Agar medium. Similarly no mycelium growth was determined at the beginning of the experiments where Minimal Agar was inoculated by strain 8 and 9.

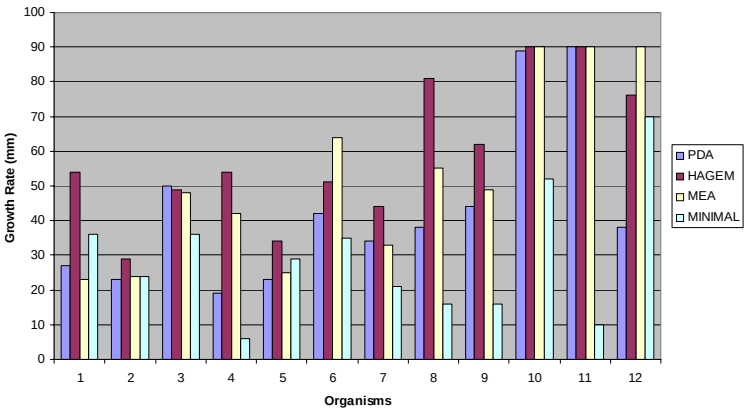

Fig. 1. Mycelial growth rate of homokaryons and heterokaryons at 27th days of incubation, (11th and 12th organisms are heterokaryons)

In many basidiomycetes, including *Hericium spp*., successful mating can be detected by the stable production of clamp connections on the hyphae in the plates. Heterokaryotic mycelia 11 and 12 showed a different mycelium growth rate, as the homokaryotic mycelia used in this study. Strain 11 in particular, when incubated in the Minimal Agar Medium could grow very slowly. Although strain 11 incubated in Hagem medium and MAE media covered the agar plate within 27 days; it could only reach to a 10 mm radial growth in the Minimal Agar Medium at the same incubation time.

The measurement of radial growth of the used homokaryotic isolates are depicted in Figure 1. The diameter of the radial growth value on the same microbiological medium is presented as a chart. Strain 3 and 10 incubated at PDA and MEA media were more active strains with higher growing rates than the others. Strain 4 growing on the MEA showed a slower growth rate until reaching to 16 mm diameter, with a gradual increase in growth rate, up to 42 mm diameter.

Ten putative homokaryons were crossed in all possible combinations by pairing up in the PDA medium, in order to produce the heterokaryons. The forty-five crosses were carried out in this experimental study, and 14 heterokaryotic mycelia were obtained.

There is a need to develop fungal strains which possess different ecophysiological properties to develop ways to effectively use genetic variation in breeding or genetic research. Those kind of preliminary isolation studies are very important and helpful in revealing the performance of new isolates[7].

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