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Mass culture of house dust mites, *Dermatophagoides farinae* and *D. pteronyssinus* (Acari: Pyroglyphidae)

Han-Il REE[1], In-Yong LEE[1], Tae-Eun KIM[1], Soung-Hoo JEON[1] and Chein-Soo HONG[2]

[1] *Department of Parasitology and* [2] *Department of Internal Medicine, College of Medicine, Yonsei University, Seoul 120–752, Korea*

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Abstract: In order to produce large amounts of antigens of *Dermatophagoides farinae* (DF) and *D. pteronyssinus* (DP), both of which are very important species as the main inhalant allergens causing allergic diseases, mass-rearing techniques of DF and DP mites were studied. A mixture of 50% fish food powder and 50% dried yeast gave the highest production of both DF and DP, showing 37.0-fold and 51.8-fold increase in number after 12 weeks, respectively. When the same amount of culture media were used, the larger surface of the rearing container gave better production rate in both cases of DF and DP, showing 188.2-fold and 200.8-fold increase, respectively in a 154 cm^2 surface container (14 cm in diameter) compared to a 79 cm^2 surface container (10 cm in diameter) after 12 weeks. Several different temperature and relative humidity conditions were compared for finding the maximum mass production. The highest production of DF mites resulted when 28°C and 64% RH were provided, showing 815-fold increase in number after 10 weeks, and followed by 28°C and 52% RH showing 773.3-fold increase after 10 weeks. In the case of DP mass rearing, the maximum production resulted when 25°C and 75% RH were given, showing 1,391.7-fold increase in number after 10 weeks, and almost the same production resulted under conditions of 28°C and 64% RH giving 1,385-fold increase in number after 10 weeks. When a 154 cm^2 surface container was used, the optimum amount of culture media was 50 g, and satisfactory result was obtained when the culture was started with 1,500 seed mites. During 20 weeks' observation period, the peak in number was obtained after 10 weeks of the culture in all test groups of DF and DP, and thereafter the number decreased.

INTRODUCTION

The most important mites in house dust are *Dermatophagoides pteronyssinus* (DP) and *D. farinae* (DF), which have world-wide occurrence and are responsible for the production of house dust allergen, a common cause of asthma and allergic rhinitis (Bronswijk and Sinha, 1971; Spieksma, 1988). It is known that both DF and DP mites are the main cause of allergic diseases in Korea as well. Kim *et al.* (1988) measured the amount of mite allergens from 20 homes in Seoul and found both DF and DP allergens from most of the study samples. Hong (1991) reported that 47.4% of 76 allergic patients had specific IgE against both DF and DP, 18.4% against DF alone and 4% against DP alone. Hong and Lee (1992) collected 340 house dust samples in Seoul and detected group I allergens (*Derf* I and *Derp* I) from 96% of the samples, of which both aller-

gens were found in 59% of the samples. Ree *et al.* (1997) studied the fauna and geographical distribution of house dust mites at 10 different locations in Korea and reported that DF and DP mites co-existed in most homes and the predominant species, either DF or DP, was different by location.

The production of the coarse and/or purified antigen of DF and DP is an essential task not only for diagnosis and treatment of allergic patients, but also for studies on properties and/or characteristics of the antigen proteins of these mites. Nevertheless, the development of mass culture techniques of DF and DP have been hitherto little studied, and these mites have been cultured on a rather small scale in many institutions (Oshima and Sugita, 1966; Larson *et al.*, 1969; Hall *et al.*, 1971; Shamiyeh *et al.*, 1971). Sasa *et al.* (1970) studied how to culture DF and some other mites on a large scale, and Miyamoto *et al.* (1975) reported a method for mass culture of DP. The objective of the present study was to find out the most efficient method of DF and DP mass culture for large quantity of antigen production.

Materials and Methods

The culture method was principally followed by Miyamoto *et al.* (1975), and constant humidity conditions were maintained by the method of Solomon (1952). Two different sized plastic containers were used, a small container (10 cm in diameter) with culture media and a large container (16 cm in diameter) with a saturated NaCl solution about 1.5–2 cm deep. The seed mites were put into the culture media and this container without a lid was placed in the middle of the large container, the lid of which was covered, so that 75% of the relative humidity was kept inside. The culture media used were a mixture of the powdered laboratory mouse food and dried yeast, and the insectary was kept at 25°C with 14 : 10 hours of light : dark condition.

In order to find out the most efficient culture media, several different media were compared under the same conditions. The tested media were (1) dried yeast, (2) powdered laboratory mouse food, (3) powdered pig food, (4) powdered fish food, (5) a mixture of 50% mouse food and 50% yeast, (6) a mixture of 50% pig food and 50% yeast, (7) a mixture of 50% fish food and 50% yeast, (8) a mixture of 50% mouse food, 25% fish food and 25% yeast. The laboratory mouse food manufactured by Samyang Co., Ltd., was made of raw materials: corn, wheat, soybean, fish powder, okkuluten, soybean oil, limestone, salt, potassium chloride, vitamins, minerals, etc., the main components of which are 23.2% of crude protein, 4.0% of crude fat, 6.0% of crude fiber, 10% of crude ash, 0.6% calcium and 0.4% of phosphine. The pig food manufactured by Miwon Co., Ltd., was made of raw materials: corn, soybean, confectioneries, fish powder, pig fat, glucose, potassium chloride, wheat, yeast, organic acids and others, the main components of which are 18% of crude protein, 5% of crude fat, 5.5% of crude fiber, 8.0% of crude ash, 0.7% of calcium and 0.5% of phosphine. The fish food manufactured by Miwon Co., Ltd., was made of raw materials: miscellaneous fish powder, soybean, wheat, horse dung, yeast, sodium phosphate, salt, vitamins (A, D_3, C, E, K_3, B_1, B_2, B_6 and B_{12}), folic acid, okkuluten, choline chloride, biotin, and minerals (Fe, Cu, Co, Mg, Zn, I, Mn), the main components of which are 43% of crude protein, 3% of crude fat, 4% of crude fiber, 16% of crude ash, 1.6% of calcium and 1.3% of phosphine. The yeast used was Ebioze® powder manufactured by Samil Pharmacy Co., Ltd.

The size of the culture container determines the surface area of the media when the same quantity of media is used, and most mites inhabit the upper part of the culture media. Containers of two sizes, one 10 cm in diameter (79 cm^2 surface) and the other 14 cm in diameter (154 cm^2 surface),

were compared. The culture media were 50% fish food and 50% yeast mixture, and the temperature and relative humidity were maintained at 25°C and 75% RH.

The optimal conditions of temperature and relative humidity were also compared. The tested temperature and relative humidity were (1) 25°C, 52%, (2) 25°C, 64%, (3) 25°C, 75%, (4) 28°C, 52%, (5) 28°C, 64% and (6) 28°C, 75%. The culture medium used was a mixture of 50% fish food and 50% yeast, and the 154 cm^2 surface container was used. The required humidity was maintained with the saturated solution of NaCl for 75%, NH_4NO_3 for 64% and $NaHSO_4$ for 52%.

Two different amounts of culture media were compared, 50 g and 25 g in the 154 cm^2 surface container. Conditions of the culture were 25 temperature, 75% relative humidity and the culture medium was 50% fish food and 50% yeast mixture.

The procedure of the mite harvest from culture media was as follows. The culture medium with mites was sieved through 28 mesh (600 μm opening) and 200 (75 μm opening) mesh sieves by flushing tap water. The mites mixed with the debris of the medium on the 200 mesh sieve were transferred into a 500 m*l* flask filled with saturated NaCl solution, and left for 20 minutes after being stirred. The supernatant was centrifuged with 650 g/10 min. The supernatant (pure mites) was transferred on the 200 mesh sieve and washed with tap water for 5 minutes in order to clear the NaCl solution. The pure mites were harvested on the filter paper of a Buchner funnel.

Results

The comparative study results of the different culture media are given in Table 1 and Table 2. In the case of DF culture (Table 1), the mixture of 50% powdered fish food and 50% dried yeast gave the maximum yield, showing 26.5-fold and 37.0-fold increase in number after 8 weeks and 12 weeks, respectively. The second recommendable medium was the mixture of 50% laboratory mouse food, 25% fish food and 25% yeast, giving 19.0-fold and 37.7-fold increase in number after 8 weeks and 12 weeks, respectively. In the case of DP culture (Table 2), the mixture of 50% fish food and 50% dried yeast gave the best result, showing 31.3-fold and 51.8-fold increase in number after 8 weeks and 12 weeks, respectively, and followed by the pure fish food powder, 50% mouse food-50% yeast mixture and 50% mouse food-25% fish food-25% yeast mixture giving 46.6-fold, 44.5-fold and 42.5-fold increase in number, respectively, after 12 weeks of the culture period.

When the same amount of culture media was used, the larger surface of the

Table 1. Number of *Dermatophagoides farinae* in different culture medium (50 g) at 25°C and 75% RH.

Culture media	No. of mites in 50 g culture media							
	0 week		4 weeks		8 weeks		12 weeks	
	Ave.*	Fold	Ave.*	Fold	Ave.*	Fold	Ave.*	Fold
Yeast	1,300	1	2,066	1.6	18,612	14.3	34,514	26.5
Mouse food	1,300	1	3,883	3.0	8,258	6.4	—	—
Pig food	1,300	1	5,169	4.0	25,985	20.0	40,685	31.3
Fish food	1,300	1	9,894	7.6	23,139	17.8	34,971	26.9
Mouse food 1 : Yeast 1	1,300	1	3,436	2.6	17,032	13.1	24,742	19.0
Pig food 1 : Yeast 1	1,300	1	2,678	2.1	15,973	12.3	21,371	16.4
Fish food 1 : Yeast 1	1,300	1	9,080	7.0	34,398	26.5	48,114	37.0
Mouse food 2 : Fish food 1 : Yeast 1	1,300	1	9,655	7.4	24,745	19.0	48,971	37.7

* The average of 5 replicates.

Table 2. Number of *Dermatophagoides pteronyssinus* in different culture medium (50 g) at 25°C and 75% RH.

Culture media	No. of mites in 50 g culture media							
	0 week		4 weeks		8 weeks		12 weeks	
	Ave.*	Fold	Ave.*	Fold	Ave.*	Fold	Ave.*	Fold
Yeast	1,100	1	3,828	3.5	12,020	10.9	44,685	40.6
Mouse food	1,100	1	1,513	1.4	—	—	—	—
Pig food	1,100	1	1,260	1.1	1,656	1.5	16,457	15.0
Fish food	1,100	1	5,671	5.2	16,589	15.1	51,257	46.6
Mouse food 1 : Yeast 1	1,100	1	2,987	2.7	19,943	18.1	48,914	44.5
Pig food 1 : Yeast 1	1,100	1	4,894	4.4	12,775	11.6	37,085	33.7
Fish food 1 : Yeast 1	1,100	1	5,154	4.7	34,444	31.3	57,028	51.8
Mouse food 2 : Fish food 1 : Yeast 1	1,100	1	1,429	1.3	10,535	9.6	46,742	42.5

* The average of 5 replicates.

Table 3. Reproduction of *Dermatophagoides farinae* and *D. pteronyssinus* in 50 g culture medium (yeast 1 : fish food 1) of two different sizes of the culture container (25°C, 75% RH).

Species	Weeks	10 cm in diameter		14 cm in diameter	
		No. of mites*	Fold of increase	No. of mites*	Fold of increase
D. farinae					
	0	1,300	—	1,354	—
	4	9,080	7.0	13,540	10.0
	8	34,398	26.5	47,661	35.2
	12	48,114	37.0	244,668	188.2
D. pteronyssinus					
	0	1,100	—	1,100	—
	4	5,154	4.7	3,722	3.4
	8	34,444	31.3	43,613	39.6
	12	57,028	51.8	220,854	200.8

* The average of 5 replicates.

container gave better yields in both cases of DF and DP, as shown in Table 3. In the case of DF, there was 188.2-fold increase in number in the 154 cm^2 surface container (14 cm in diameter), whereas only 37.0-fold increase was shown in the 79 cm^2 surface container (10 cm in diameter) after 12 weeks. In the case of DP, 200.8-fold and 51.8-fold increases in number in the 154 cm^2 and 79 cm^2 surface containers, respectively, after 12 weeks were shown.

Table 4 shows the result of the comparative studies of DF culture under different temperatures and relative humidities. The highest production was obtained when 28°C and 64% RH were given, showing 815-fold increase after 10 weeks, and followed by the condition of 28°C and 52% RH showing 773.3-fold increase in number after 10 weeks. The poorest result was obtained when they were cultured at 28°C and 75% RH, showing 221.3-fold increase in number after 10 weeks. In the case of DP, as shown in Table 5, the maximum production rate was obtained when the temperature and relative humidity were maintained at 25°C and 75% RH, giving 1,391.7-fold increase in number after 10 weeks, and almost the same result was obtained at 28°C and 64% RH, giving 1,385-fold increase in number after 10 weeks. During 20 weeks' observation period of both DF and DP, the maximum increase in number was observed in week

Table 4. Comparison of *Dermatophagoides farinae* culture under different temperature and relative humidity conditions in 50 g culture medium (50% fish food + 50% yeast) in the container of 154 cm^2 surface.

Temperature & humidity		Number of mites in 50 g medium (unit: 1,000)							
		0 wk	2 wks	4 wks	8 wks	10 wks	12 wks	16 wks	20 wks
25°C, 52%	Ave.*	1.5	11	144	415	717.5	453	834	34
	Fold	1	7.3	96	276.6	478.3	302	556	22.7
25°C, 64%	Ave.*	1.5	18	168	440	605	348	83	13
	Fold	1	12	112	293.3	403.3	232	55.3	8.7
25°C, 75%	Ave.*	1.5	20	174	430	877.5	340	166	18
	Fold	1	13.3	116	286.7	585	226.7	110.7	12
28°C, 52%	Ave.*	1.5	7	157	694	1,160	782	302	5
	Fold	1	4.7	104.7	462.7	773.3	521.3	201.3	3.3
28°C, 64%	Ave.*	1.5	4.4	189	720	1,222.5	529	103	1.6
	Fold	1	2.9	126	480	815	352.7	68.7	1.1
28°C, 75%	Ave.*	1.5	9	186	180	332	306	195	32
	Fold	1	6	124	120	221.3	204	130	21.3

* Average of 5 duplicates.

Table 5. Comparison of *Dermatophagoides pteronyssinus* culture under the different temperature and relative humidity conditions in 50 g culture medium (50% fish food + 50% yeast) in the container of 154 m^2 surface.

Temperature & humidity		Number of mites in 50 g medium (unit: 1,000)							
		0 wk	2 wks	4 wks	8 wks	10 wks	12 wks	16 wks	20 wks
25°C, 52%	Ave.*	1.5	25	207	657	1,505	1,391	1,400	761
	Fold	1	16.7	138	438	1,003.3	927.3	933.3	507.3
25°C, 64%	Ave.*	1.5	26	215	683	1,240	824	1,012	645
	Fold	1	17.3	143.3	455.3	826.7	549.3	674.7	430
25°C, 75%	Ave.*	1.5	26	239	920	2,087.5	1,162	1,152	872
	Fold	1	17.3	159.3	613.3	1,391.7	774.7	768	581.3
28°C, 52%	Ave.*	1.5	13	243	853	1,665	1,285	937	609
	Fold	1	8.7	162	568.7	1,110	856.7	624.7	406
28°C, 64%	Ave.*	1.5	10	311	1,061	2,077.5	1,297	1,028	362
	Fold	1	6.7	207.3	707.3	1,385	864.7	685.3	241.3
28°C, 75%	Ave.*	1.5	20	246	768	1,208	776	518	117
	Fold	1	13.3	164	512	805.3	517.3	345.3	78

* Average of 5 duplicates.

10 in all test groups of DF and DP, and thereafter the number of mites kept decreasing steadily.

Table 6 shows the study result for the optimum number of seed mites (introduced mites at the beginning) and the optimum amount of culture media when 154 cm^2 surface container (14 cm in diameter) was used. In the case 25 g of culture medium was used, the numbers of mites in week 10 were 1052.5, 1095 and 1069.5 in the groups of 1500, 3000 and 4500 seed mites, respectively, which means that the increase of seed mites does not increase the production rate of DP mites. In the case 50 g of culture medium was used, the numbers of mites after 10 weeks were 1401, 1556 and 1532 in the groups of 1500, 3000 and 4500 seed mites, respectively, which was the same result as in the test of 25 g of culture medium. A slightly higher increase in number was obtained in 50 g of culture media than in 25 g of culture media irrespective of the number of

Table 6. Propagation rate of *Dermatophagoides pteronyssinus* in culture medium (50% fish food + 50% yeast) under the condition of 25°C and 75% RH. The containers used were all 14 cm in diameter.

	in 25 g culture medium (unit: 1,000)					in 50 g culture medium (unit: 1,000)				
	0 wk	4 wks	6 wks	8 wks	10 wks	0 wk	4 wks	6 wks	8 wks	10 wks
Ave.*	1.5	36.8	278.6	828.5	1,052.5	1.5	56.0	314	968	1,401
Fold	1	24.3	185.7	552.3	701.7	1	37.3	209.3	645.3	934.0
Ave.*	3	86	422	1,079	1,095	3	73	464	990	1,556
Fold	1	28.7	140.7	359.7	365.0	1	24.3	154.7	330	518.7
Ave.*	4.5	132.0	497.0	984.0	1,069.5	4.5	102.0	635	1,341	1,532
Fold	1	29.3	110.4	218.7	237.6	1	22.7	141.1	298.0	340.4

* Average of 5 duplicates.

seed mites that were introduced.

In conclusion, the maximum yield of both DF and DP mites could be obtained when 50 g of the mixture of fish food powder (1 part) and dried yeast (1 part) were used for the culture medium in a container of 154 cm^2 surface (14 cm in diameter) and when temperature and relative humidity were kept at 28°C and 64% RH for DF and 25°C and 75% RH for DP. The maximum harvest was obtained in week 10 for both DF and DP.

Discussion

The occurrence of mites in house dust habitats has been related to a number of physical and climatic factors, in particular, temperature and humidity (Arlian, 1976). House dust mites can passively and actively absorb water from unsaturated air, and on the other hand, mites also constantly and simultaneously lose water from the body surface by transpiration or through processes associated with feeding, reproduction, defection and excretion. Feeding rates of DF and DP are significantly higher at ambient water vapor activities above the critical equilibrium activity than below it (Arlian, 1977). Koekkoek and Bronswijk (1972) reported that an optimal development of DP was shown at a temperature of 25°C, and after 8 weeks the number of mites reared at 30°C was 40% of the number at 25°C and 15% of the number at 20°C. Sasa *et al.* (1970) studied mass culture of DF. They fixed the temperature at 25°C and compared different humidity conditions (8–18%) which were controlled by adding water directly to the culture media, and it was found that the highest recovery of DF mites was obtained at 12% humidity. Waki and Matsumoto (1973a) compared the different temperature and humidity conditions for the reproduction of DF mites, and the maximum reproduction was obtained at 25 and 57% after 23 weeks. Miyamoto *et al.* (1975) reported that the highest yield of DP mites was obtained with the relative humidity at 75% and the temperature at 25°C. Matsumoto *et al.* (1986) compared the effect of different humidity conditions (86, 76, 61 and 36% RH) on life cycles of DF and DP by observing individual development and the shortest duration of the development from an egg to an adult was obtained at 76% RH and 25°C for both species. In the case of DF, this result differed from that of Waki and Matsumoto (1973a). The results of the present study showed that the optimum condition of temperature and relative humidity was 28°C and 64% RH for DF and 25°C and 75% RH for DP. In the case of DP, the result coincided with that of Miyamoto *et al.* (1975).

Selection of the culture media is also one of the important factors for successful mass culture of house dust mites. Sasa *et al.* (1970) reported that the yield from Daphnia (water flea) powder or Albimine

powder was poor, and the powdered laboratory mouse food for DF culture gave the maximum yield. Waki and Matsumoto (1973b) found that the highest number of DF mites was obtained when 5% of lard was added to the medium of fatless food and yeast. Matsumoto (1975) compared various kinds of lipids for the breeding of DF mites and found that the optimum amount (4–6%) of lipids such as cotton oil, peanut oil, butter, lecithin and mono-olein was essential for higher production. Miyamoto *et al.* (1975) found that the mixture of 2 parts of powdered laboratory animal food, 2 parts of dried yeast and 1 part of fish food powder was most appropriate for DP mass culture. The result of the present study showed that the mixture of 1 part of fish food powder and 1 part of dried yeast gave the highest yield of both DF and DP mites. The mixture of 2 parts of laboratory mouse food, 1 part of fish food and 1 part of yeast also showed the same result as the mixture of 1 part of fish food and 1 part of yeast in the case of DF culture. However, pure isolation of mites from the debris of culture media was not possible when the laboratory mouse food powder was used. On the other hand, when the mixture of fish food powder and dried yeast was used, pure mites could be easily separated from the culture media by centrifuging with 650 g for 10 minutes. The process of thorough stirring by upside-down media and mites at 2 days' intervals was neccessary for keeping the constant humidity and even distribtion of mites in the medium. The authors did not try to test the culture media proposed by Waki and Matsumoto (1973b) and Matsumoto (1975), because the procedure of defatting fish food powder and adding 4–6% of oils seemed not to be practical for the mass culture of house dust mites, DF and DP.

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