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Development of mass rearing technique of *Tyrophagus putrescentiae* (Acari: Acaridae) found in house dust

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Abstract: A storage mite, *Tyrophagus putrescentiae*, is recently known to be widely distributed in Korea, being commonly found in house dust, and may, therefore, be allergenically important. The purpose of this study was to develop mass rearing techniques for supplying a large quantity of allergens. The laboratory mouse food powder gave the highest yield, showing 1,251.5-fold increase in number after 10 weeks, and the mixed powder of laboratory mouse food and yeast (1:1) also gave same level of the production (1,203.1-fold increase in week 10). Several different combinations of temperature and relative humidity conditions were compared, and the maximum propagation was obtained at 25°C and 64% RH, showing 960-fold increase in number. When the same amount of culture media was used, the size of the culture container did not significantly influence the quantitative yield of *T. putrescentiae* mites.

Key words: mass rearing, *Tyrophagus putrescentiae*, storage mite, house dust

INTRODUCTION

It is well known that house dust mites cause allergenic diseases such as asthma and rhinitis, and *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* are the most important species of the house dust mites throughout the world (Bronswijk and Sinha, 1971). Several species of storage mites occur in significant numbers, particularly when relative humidity is high. Though the role of these storage mites as allergens is less clear, antigenic and allergenic properties of *Tyrophagus putrescentiae* were studied (Arlian *et al.*, 1984a) and some antigenic and allergenic determinants were shared by *D. farinae* (Arlian *et al.*, 1984b). Ree *et al.* (1997a) reported that *T. putrescentiae* was the third predominant species found in house dust throughout Korea. Therefore, it is presumed that this species also would be one of the main allergens which cause allergenic diseases in Korea.

The production of purified antigens of *T. putrescentiae* is an essential task for finding whether this species causes allergenic diseases and then for studying properties and characteristics of the antigen proteins. The objective of the present study was to develop mass rearing techniques of this species for supplying large quantity of the antigen.

MATERIALS AND METHODS

At the begining of the study, the culture method was principally followed the method of Miyamoto *et al.* (1975), and maintenance of the constant relative humidities was followed the method of Solomon (1952).

The following culture media were compared:

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(1) dried yeast, (2) laboratory mouse food, (3) swine food, (4) fish food, (5) a mixture of mouse food and yeast (1:1), (6) a mixture of swine food and yeast (1:1), (7) a mixture of fish food and yeast (1:1), (8) a mixture of mouse food and swine food (1:1), and (9) a mixture of mouse food and fish food (1:1). All culture media were powdered. 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 and minerals, 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 0.5% 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 tested was Ebioze powder manufactured by Samil Pharmacy Co., Ltd.

The optimal conditions of temperature and relative humidity were evaluated. The following combinations of temperature and relative humidity were compared: (1) 25°C, 52% RH, (2) 28°C, 52% RH, (3) 25°C, 64% RH, (4) 28°C, 64% RH, (5) 25°C, 75% RH and (6) 28°C, 75% RH. The culture medium used was a mixture of fish food and yeast (1:1), and the 154 cm^2 surface container was used. The required relative humidity was maintained by putting the saturated solution of NaCl (for 75% RH), NH_4NO_3 (for 64% RH) and $NaHSO_4$ (for 52% RH) in the large container (Fig. 1).

Three different sizes of the containers, 10 cm in diameter (79 cm^2 surface), 14 cm in diameter (154 cm^2 surface), and 17 cm in diameter (227 cm^2 surface) were compared. Fifty gram of laboratory animal food and yeast mixture (1:1) were put in each container together with seed mites, and the temperature and relative humidity were maintained at 25°C and 75% RH.

For mite counts, 0.01 g of the culture media

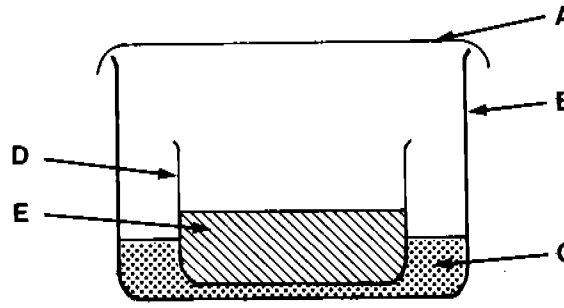

Fig. 1. Culture method of mites by using large and small containers. A: lid, B: large plastic container (20 cm in diameter), C: saturated solution of NaCl, Na_4HO_3 or $NaHSO_4$, D: plastic culture container (14 cm in diameter), E: culture medium and mites.

was taken, immediately after being stirred thoroughly, from each culture container, and the mites were directly counted under a stereo-microscope with two week intervals. The procedure of the mite harvest from culture media was as follows. The culture medium with mites was sieved through a 28 mesh sieve (600 μm opening) and a 200 mesh sieve (75 μm opening) by flushing tap water. The mites mixed with the debris of the medium on the 200 mesh sieve were transferred into a 500 ml 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 onto 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

Among 9 different media and/or different combination of the media tested for mass rearing of *Tyrophagus putrescentiae* mites, the maximum yield was obtained in the mouse food powder, showing 1,251.5-fold increase in number after 10 weeks. The mixture of yeast and laboratory mouse food (1:1) also gave almost same production showing 1,203.1-fold increase in number after 10 weeks. The least propagation was shown in the fish food powder, being increased only 623.8-fold and 367.7-fold in number after 8 and 10 weeks,

Table 1. Comparative mass production of *Tyrophagus putrescentiae* in the different culture media (50 g) under the condition of 25°C and 75% RH

Culture medium		No. of mites (unit: 1000)					
		0 wk	4 wks	6 wks	8 wks	10 wks	12 wks
Yeast	Ave.[a]	1.3	35	373	881	973	705
	Fold	1	26.9	286.9	677.7	748.5	542.3
Mouse food	Ave.[b]	1.3	227	859	1,359	1,627	1,050
	Fold	1	174.6	660.8	1,045.4	1,251.5	807.7
Swine food	Ave.[a]	1.3	156	780	760	1,400	378
	Fold	1	120	600	584.6	1,076.9	290.8
Fish food	Ave.[a]	1.3	205	501	811	478	125
	Fold	1	157.7	385.4	623.8	367.7	96.2
Yeast + Mouse food (1:1)	Ave.[b]	1.3	256	704	1,196	1,564	1,215
	Fold	1	196.9	541.5	920	1,203.1	934.6
Yeast + Swine food (1:1)	Ave.[b]	1.3	256	425	1,042	1,323	1,002
	Fold	1	196.9	326.9	801.5	1,017.6	770.8
Yeast + Fish food (1:1)	Ave.[b]	1.3	99	653	961	1,121	959
	Fold	1	76.2	502.3	739.2	862.3	737.7
Mouse food + Swine food (1:1)	Ave.[a]	1.3	156	745	826	960	1,011
	Fold	1	120	573.1	635.4	738.5	777.7
Mouse food + Fish food (1:1)	Ave.[a]	1.3	150	501	878	915	865
	Fold	1	115.4	385.4	675.4	703.8	665.4

[a]The average of 3 replicates; [b]The average of 11 replicates.

respectively (Table 1).

The result of the comparative studies for finding the most suitable temperature (°C) and relative humidity (% RH) is shown in Table 2. The highest production was obtained when 25°C and 64% RH were given, showing 960-fold increase in number after 11 weeks, and followed by the condition of 25°C and 75% RH, showing 922.3-fold and 935.4-fold increase in number after 9 and 13 weeks, respectively. The poorest yield was obtained when they were cultured at 28°C and 52% RH, showing 363.1-fold increase after 15 weeks, followed by the conditions of 28°C, 64% RH and 25°C, 52% RH, showing 610.8-fold increase after 13 weeks and 645.4-fold increase after 9 weeks, respectively.

When the same amount (50 g) of culture media was used, the surface areas of the container were not important factors for mass rearing of *T. putrescentiae* mites, as shown in Table 3. In the case that 79 cm^2 surface area (10 cm in diameter) of the container was used, 860.8-fold increase in number was shown after 8 weeks; in the case of the 154 cm^2 surface area (14 cm in diameter), 924.6-fold increase after 10 weeks was shown; when the surface area of 227 cm^2 (17 cm in diameter) was used, 1,250.8-fold increase in number after 10 weeks was shown. The ratio of the yield among 79 cm^2, 154 cm^2 and 227 cm^2 surface containers were 1:1.1:1.5.

DISCUSSION

Selection of the culture media is one of the very important factors for successful mass rearing of house dust/storage mites. Sasa *et al.* (1970) reported that a laboratory mouse food powder was found to be fitted for the culture of *D. farinae*, *T. putrescentiae* and *Aleuroglyphus ovatus*. Ree *et al.* (1997b),

Table 2. Comparative mass production of *Tyrophagus putrescentiae* under the different temperature and humidity conditions in 50 g of the culture medium (Laboratory mouse food:Yeast, 1:1)

Temp. Humid		No. of mites (unit: 1,000)						
		0 wk	4 wks	6 wks	9 wks	11 wks	13 wks	15 wks
25°C	Ave.[a]	1.3	11	207	839	757	734	277
52%	Fold	1	8.5	159.2	645.4	582.3	564.6	213.1
28°C	Ave.[a]	1.3	40	252	165	278	415	472
52%	Fold	1	30.8	193.8	126.9	213.8	319.2	363.1
25°C	Ave.[a]	1.3	185	686	1,096	1,248	1,082	903
64%	Fold	1	142.3	527.7	843.1	960	832.3	694.6
28°C	Ave.[a]	1.3	220	522	627	359	794	119
64%	Fold	1	169.2	401.5	482.3	276.2	610.8	91.5
25°C	Ave.[a]	1.3	297	608	1,199	1,020	1,216	594
75%	Fold	1	228.5	467.7	922.3	784.6	935.4	456.9
28°C	Ave.[a]	1.3	302	287	513	809	424	82
75%	Fold	1	232.3	220.8	394.6	622.3	326.2	63.1

[a]The average of 5 replicates.

Table 3. Comparative mass rearing of *Tyrophagus putrescentiae* in the different sizes of the container in 50 g of culture media (Laboratory mouse food:Yeast, 1:1) under the condition of 25°C and 75% RH

Surface area (cm^2)		No. of mites (unit: 1,000)					
		0 wk	4 wks	6 wks	8 wks	10 wks	12 wks
79	Ave.[a]	1.3	241	733	1,119	1,040	796
	Fold	1	185.4	563.8	860.8	800	612.3
154	Ave.[a]	1.3	396	695	1,110	1,202	661
	Fold	1	304.6	534.6	853.8	924.6	508.5
227	Ave.[b]	1.3	329	776	—	1,626	379
	Fold	1	253.1	596.9	—	1,250.8	291.5

[a]The average of 10 replicates; [b]The average of 5 replicates.

however, found that the mixture of fish food and yeast (1:1) gave the highest yield of both *D. farinae* and *D. pteronyssinus*. The present study showed that either the laboratory mouse food powder or the mixed powder of mouse food and yeast (1:1) gave the equally good result in propagation of *T. putrescentiae* mites.

The temperature and relative humidity are also an important factor for mass culture of mites, and temperature and humidity are closely related, working complementary each other. Sasa *et al.* (1970) reported that the highest recovery of mites was obtained by adding water to the culture media to 12% in case of *D. farinae* and 15% in case of *T. putrescentiae* at 25°C. Miyamoto *et al.* (1975) reported that the highest yield of *T. putrescentiae* was obtained with 75% RH and the temperature at 25°C. Ree *et al.* (1997b) reported that the maximum yield was obtained under the condition of 25°C and 75% RH in case of *D. pteronyssinus* and 28°C and 64% RH in case of *D. farinae*. It is obvious that *T. putrescentiae* and *D. pteronyssinus* prefer to

the higher humidity condition, compared to *D. farinae* mites. In the present study, the highest yield was obtained at 25°C and 75% RH, and the equally good yield at 25°C and 64% RH.

The size of the culture container determines the surface area of the media when the same quantity of media is used, and it is presumed that mites prefer to inhabit near surface of the culture media. In fact, Ree *et al.* (1997b) found that the larger surface of the container (14 cm in diameter, 154 cm^2) gave much higher yields in both cases of *D. farinae* and *D. pteronyssinus* compared to the smaller surface of the container (10 cm in diameter, 79 cm^2), showing that mite propagation in the larger container was 3.9-5.1 times higher compared to the smaller one in both species. However, the present study on mass culture of *T. putrescentiae* resulted little difference among three sizes of the container as shown in Table 3. This result probably indicates that *T. putrescentiae* mites inhabit not only at near surface (upper part) but also preferably penetrate deep into the culture medium when they are over-crowded.

The peak in number was obtained after 10 weeks of the culture in most test groups, and thereafter the number decreased, which would probably be resulted from the fact that the obtimum number of mites in 50 g of the culture medium reaches in week 10 and becomes over-crowded thereafter. In conclusion, the highest yield of *T. putrescentiae* mites could be obtained when 50 g of either the laboratory mouse food powder or the mixed powder of laboratory mouse food and yeast (1:1) were used under the condition of 25°C and 75% RH. The recommendable period of the harvest is in week 9-10.

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=초록=

집먼지에 서식하는 긴털가루진드기(진드기목: 가루진드기과)의 대량 사육방법 개발

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저장 곡류 해충으로 알려져 있는 긴털가루진드기(*Tyrophagus putrescentiae*)가 우리 나라 가옥 내 집먼지 서식 진드기류 중 제 3의 우점종으로 밝혀짐에 따라 알레르기성 질환을 야기하는 원인 항원으로 작용할 가능성이 높아져 연구의 대상이 되고 있다. 본 연구에서는 진단, 치료 및 면역학적 연구에 필요한 다량의 항원을 공급하기 위하여 대량 사육방법의 개발을 시도하여 다음과 같은 결과를 얻었다. 긴털가루진드기를 대량 사육하는데 가장 적합한 사육배지로는 실험동물사료 분말로 사육 10주 후 1,251.5배의 개체 증가를 보였고, 효소분말과 실험동물 사료분말의 혼합배지(1:1)에서도 사육 10주 후 1,203.1배의 개체 증가를 보여 거의 같은 성적을 보였다. 사육조건으로 가장 적합한 온도와 상대습도는 25°C, 64% RH로서 9주 후 960배로 가장 높은 증식결과를 보였고, 25°C와 75% RH에서도 935.4배의 개체 증가로 거의 동일한 결과를 나타냈다. 사육용기 표면적을 79 cm^2, 154 cm^2 및 227 cm^2로 사육 비교한 결과 각각 860.8배, 924.6배 및 1,250.8배의 개체 증가를 보여 현저한 차이는 보이지 않았다.

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