FULL PAPER *Internal Medicine*

Study of Lipid in the Ear Canal in Canine Otitis Externa with *Malassezia pachydermatis*

Akira MASUDA[1,2], Takeshi SUKEGAWA[2], Naoe MIZUMOTO[1], Hiroyuki TANI[1], Tadashi MIYAMOTO[1], Kazumi SASAI[1] and Eiichiroh BABA[1]*

[1]*Veterinary Internal Medicine, Department of Veterinary Medicine, Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, Sakai, Osaka 599–8531 and* [2]*Masuda Dog & Cat Hospital, Hirakata, Osaka 573–1111, Japan*

(Received 8 May 2000/Accepted 3 August 2000)

ABSTRACT. An epidemiological investigation of 120 canine otitis externa cases in 1,370 dogs was done on the incidence rate, ear pinna shapes, breeds and their relationships. Eighty-five cases (12.6%) in 672 dogs with pendulous ears and 35 cases (5.0%) in 698 dogs with erect ears had otitis externa, and the difference between them was significant ($P<0.05$). Ninety-five auditory cerumen specimens were cultured for *Malassezia pachydermatis* (*M. pachydermatis*) and analyzed for concentrations of major fatty acids. Although rates of cases positive for *M. pachydermatis* in both ear pinna shapes were almost the same, i.e. 55.2% in the pendulous group and 53.6% in the erect group, the average total fatty acid level of the pendulous ear group was significantly ($P<0.05$) higher than that in the erect ear group after dismissing extraordinary levels in the Siberian husky. Isolated *M. pachydermatis* strains were examined for the effects of fatty acid supplementation on their growth. The majority of the strains utilized fatty acids and grew faster in fatty acid supplemented broth. These results suggest that *M. pachydermatis*, the predominant causative agent of canine otitis externa, prefers the auditory canal of dogs with lipid-rich earwax and grows fast, but growth strongly depends upon the canine breed.

KEY WORDS: canine, fatty acid, lipid, *Malassezia pachydermatis*, otitis externa.

J. Vet. Med. Sci. 62(11): 1177–1182, 2000

In small animal clinics, many fatal diseases have been eradicated up to the present owing to advances in prevention and treatment. The comparative rate of slight illnesses such as skin diseases has increased recently [10], so that non-fatal but chronic diseases must be studied from now. *Malassezia pachydermatis* has been isolated in animals more frequently than human beings [11], and experiments on inducing otitis externa with this organism have been conducted [15]. The yeast belonging to genus *Malassezia* has been recognized as one of the dermal bacterial flora [3]. Seven species have been identified–*M. pachydermatis, M. furfur, M. sympodialis, M. globosa, M. obtusa, M. restricta* and *M. sloofiae* [5]- and each species clearly apears to be related to the corresponding host. For instance, *M. furfur* is one of the most predominant causative agents of skin diseases in man, and *M. pachydermatis* has been isolated mainly in dogs [2, 12]. The relationship between *Malassezia* species and host animals remains unknown. The mechanism by which the incidence of otitis externa is closely related with ear shapes and breeds [9, 13, 14] is also not clear. Many apocrine glands are distributed in the canine auditory canal, and the density of the glands varies from breed to breed [14].

First, an epidemiological investigation on the incidence of canine otitis externa in relation to ear shapes and breeds was conducted, and then earwax specimens from canine otitis externa cases were subjected to quantitative analysis of levels of major fatty acids to find out the relationship between lipid and the incidence of otitis externa with regard to breeds and ear pinna shapes. The effects of fatty acids on the growth of *M. pachydermatis* were examined by using strains isolated in the present study.

* CORRESPONDENCE TO: BABA, E., Veterinary Internal Medicine, Department of Veterinary Medicine, Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, 1–1 Gakuencho, Sakai, Osaka 599–8531, Japan.

MATERIALS AND METHODS

Clinical surveillance: The epidemiological relationship between the incidence rates of otitis externa and dog breeds and ear shapes was surveyed in an animal hospital through one summer season (May to October, 1997) when otitis externa occurred frequently. In this study, otitis externa was clinically diagnosed by the appearance of pruritus of the ear, rubefacient, congestion and swelling in the auditory canal, purulent discharge or excessive ear wax. The affected dogs were 120 dogs from a total of 1,370 clients. The clients were divided into the pendulous ear group and the erect ear group, and the individual data, disease history, and physical examination data for these cases were recorded.

Sampling ear cerumen and recognition of M. pachydermatis: Among 120 cases, dogs under medication were excluded from the examination to avoid specimens for fatty acid analysis which contained impurities. The cerumen sample was collected from the ear canal of each dog with a sterilized stainless steel scraper. Enough ear cerumen samples for the quantitative examination of fatty acids were obtained from 95 dogs. A 10 mg sample of the cerumen was measured with a standardized bacteriological platinum loop, and suspended in 1 m*l* of phosphate bufferred solution (PBS, pH 7.4). In order to confirm *M. pachydermatis* infection, a part of each PBS suspension was spread on a Sabouraud's dextrose agar plate and incubated at 37°C for 3 to 5 days. Yeast colonies were identified. *M. pachydermatis* is cultured to

form a small colony with a dry ash-colored surface, which can be easily identified from the colony shape and microscopic characteristics. When bacterial colonies were grown, they were determined bacteriologically by way of precaution.

Fatty acids levels in the ear cerumen: The PBS suspensions made from 95 ear cerumen specimens were measured for fatty acids. According to the reference [8], concentrations of myristic (14:0), palmitic (16:0), margaric (17:0), linoleic (18:0), oleic (18:1), and stearic (18:2) acids were analyzed with modification. Benzene (400 μl) was added to each PBS suspension (100 μl), and the benzene portion including extracted fatty acids was submitted to gas-chromatographic analysis of the fatty acids. A capillary type gas chromatographer[a] with a siliconized metallic column[b] was used, and the retention times and fatty acid levels were compared with the standard chemicals[c]. The oven temperature was set at 150°C followed by intentional ascent to 240°C.

Effects of fatty acids on growth of M. pachydermatis: Two representative strains, strains A and B, were used in the preliminary experiment on growth in the presence of exogenous oleic and linoleic acid. Ten mg of each of the cultured yeasts on Sabouraud's dextrose agar plates was suspended in 10 m*l* of Sabouraud's dextrose broth and 10 μl of this suspension was used for inoculations. Before inoculation, 0.01% (v/v) of fatty acid at the final concentration was added to Sabouraud's dextrose broth containing 0.2% (w/v) polyoxyethylene lauryl ether[c], a detergent for their easy incorporation into the medium in a soluble form, and then incubated for one week at 37°C. Growth was followed by the OD value measurement (500 nm) of the culture broth by means of a spectrophotometer, and finally 3 days incubation was determined as the most suitable incubation period. Afterward, 42 strains purely isolated from the present samples were tested for the utilization of fatty acids. As mentioned above, suspensions and Sabouraud's dextrose broth containing 0.2% (w/v) of polyoxyethylene lauryl ether with 6 fatty acids referred to in the examination of the levels in the ear cerumen [8] were prepared, and growth after 3 days incubation was recorded as OD values.

Statistical analyses: Significant ($P<0.05$) differences between groups in the incidence of otitis externa and the detection of yeasts were determined by application of the chi-square test. Total levels of fatty acids were submitted to analyze significant ($P<0.05$) difference among ear pinna shapes and canine breeds by Student's t test.

RESULTS

Clinical surveillance data: Among 1,370 dogs seen during the present epidemiological investigation, 85 (12.6%) were affected with otitis externa in 672 dogs with pendulous ears, whereas 35 cases (5.0%) were in 698 dogs with erect ears (Table 1). The incidence in the two groups differed significantly ($P<0.05$). Pug (11/31: 35.5%), Poodle (10/38: 36.3%) and Maltese (20/96: 20.8%) breeds had high incidences of otitis externa in the pendulous ear group, whereas in the erect ear group West highland white terrier (3/5: 60%), Siberian husky (3/24: 12.5%), Shetland sheep dog (5/54: 9.8%) and Yorkshire terrier (7/73: 9.6%) breeds had larger numbers of otitis cases than the others.

Malassezia counts and fungal culture: Among 120 dogs clinically diagnosed with otitis externa, 95 cases with otitic cerumen specimen enough for the present quantitative measurement were selected for the culture. They consisted of 58 males and 37 females, ranging in age from 3 weeks to 17 years old. The relations between ear types, incidence of otitis externa, and numbers of positive cases for *M. pachydermatis* on the agar plates are shown in Table 2. *M. pachydermatis*-positive cases accounted for more than 10^5 CFU per gram of the original samples. A clear difference between the pendulous ear group and the erect ear group was found in the incidence of otitis externa, but there was no significant difference between the two ear types in cases positive for *M. pachydermatis* in culture. The pendulous ear group appeared more susceptible to *M. pachydermatis* than the erect ear group, but there was no significant deference.

Fatty acid levels in ear cerumen specimens: The ear cerumen specimens contained high levels of fatty acids. Averages of total fatty acid levels in the specimens are shown in Table 2 for every breed. The samples from many breeds in the pendulous ear group contained higher fatty acid levels than those from the erect ear breeds, except for the Pug in the pendulous ear breeds and the Siberian husky in the erect ear ones. Although no significant differences in the average levels were found among those breeds because of the wide variety of individuals, after dismissing extraordinary levels in the Siberian husky, the average level (331.7 μM) in the whole pendulous ear group (91.4 μM) was significantly ($P<0.05$) higher than that in the whole erect ear dogs.

The level of each level of the 6 fatty acids is shown in Fig. 1 in order of the concentration. Myristic acid was detected in a small number of samples, but very high levels were noticed in the detectable cases. For instance, one sample each from a Labrador retriever, Siberian husky and Shih tzu showed levels at least as high as 700 μM of myristic acid. Margaric acid was also detected in a limited number of samples but at low levels. Other fatty acids were detected in almost all samples. No clear relation between the each level of fatty acid and the dog breed was noticed.

Effects of fatty acids on growth of M. pachydermatis: In the preliminary experiment on two *M. pachydermatis* isolates and two sorts of fatty acids (oleic and linoleic acid), the yeasts in the fatty acid-supplemented Sabouraud's dextrose broth started to grow after 2 days, whereas growth in non-supplemented broth was barely noticed after 4 days' incubation, and thereafter agglutination of the yeasts appeared. This agglutination made it necessary to discontinue the measurement of growth (Fig. 2). A three day incubation periods

FOOTNOTE

[a] GC-17A, Shimadzu Seisakusho Inc., Kyoto, Japan

[b] Ultra Alloy 1-15M-0.25F, Frontier Laboratories Ltd., Fukushima, Japan

[c] Wako Pure Chemical Industries Ltd., Osaka, Japan

Table 1. Incidence of otitis externa in 1,370 dogs examined

Pendulous ears				Erect ears			
		Otitis externa				Otitis externa	
Breed	No. of clients	No. of cases (%)	No. of samples[a)]	Breed	No. of clients	No. of cases (%)	No. of samples[a)]
Shih Tzu	169	10 (5.9)	8	Mixed	214	9 (4.2)	8
Mixed	113	6 (5.3)	4	Japanese dogs	150	6 (4.0)	5
Maltese	96	20 (20.8)	16	Pomeranian	85	2 (2.4)	1
Labrador Retriever	83	14 (16.9)	12	Yorkshire Terrier	73	7 (9.6)	5
Dachshund	64	6 (9.4)	4	Shetland Sheepdog	51	5 (9.8)	4
Poodle	38	10 (26.3)	7	Chihuahua	26		
Beagle	33	6 (18.2)	5	Siberian Husky	24	3 (12.5)	3
Pug	31	11 (35.5)	9	German Shepherd Dog	16		
Spaniels	14	1 (7.1)	1	Miniature Schnauzer	11		
Pekingese	6			Papillon	9		
Pointer	5			Miniature Pinscher	9		
Bernese Mountain Dog	5			Doberman Pinscher	5		
Great Pyrenes	4			West Highland White Terrier	5	3 (60.0)	2
Miniature Schnauzer	3	1 (33.3)	1	Welsh Corgi	5		
Borzoi	3			Japanese Spitz	5		
Dalmatian	1			Collie	3		
Afghan Hound	1			Australian Shepherd Dog	3		
Greyhound	1			Boxer	1		
St. Bernard	1			Boston Terrier	1		
Irish Setter	1			Alaskan Malamute	1		
				French Bulldog	1		
Total	672	85 (12.6)*	67		698	35 (5.0)	28

a) More than 10 mg of ear cerumen was sampled.
*: The incidence of otitis externa in the pendulous ear dogs was significantly ($P<0.05$) higher than that in the erect ear dogs.

was selected for examining the effects of fatty acids on early stage growth of *M. pachydermatis*. Forty-two strains purely isolated from the present samples were tested. Thirty-one of 42 strains utilized any of the fatty acids and grew faster than the non-supplemented broth, but 11 strains grew independently of additional fatty acids (Table 3). In Fig. 3 the growth levels of an example that utilized 4 fatty acids for proliferation is shown. The sorts of fatty acids utilized by *M. pachydermatis* depended on the strains isolated. Palmitic and oleic acids were likely to be preferred by *M. pachydermatis*.

DISCUSSION

Otitis externa is the most commonly diagnosed disease in dogs [4, 10]. There are many underlying causes of canine otitis externa. *M. pachydermatis* has often been isolated from otitis externa cases as the most common organism and complicating factor. In the present surveillance, 120 (8.8%) of 1,370 dogs had otitis externa, and this incidence rate is similar to recent data reported by another researcher [10]. A higher incidence of otitis externa in the pendulous ear type breeds than in the erect ear type was reported, and the difference was explained by more moisture in pendulous ears [7].

Recently, more evidence has shown that it is not only the type of ear but actual variations in the microanatomy of the external auditory wall that may predispose certain breeds to otitis externa. In one study, Cocker spaniels, Springer spaniels, and Labrdor retrievers, which are breeds that are commonly afflicted with otitis externa, were found to have significantly more apocrine glands than Greyhound [14]. The former breeds also had a higher density of hair follicles throughout the horizontal canal.

In otitis externa cases, the relationship between the dog breed and amounts of lipid of earwax samples was investigated. Amounts of fatty acids instead of lipid were analyzed quantitatively. Most of the pendulous ear type dogs had high amounts of fatty acids in the cerumen samples, but there were exceptions such as the Pug. During the surveillance, one third of Pugs that visited the hospital were suffering from otitis externa, and this incidence was the highest in the pendulous ear breeds, but the amount of total fatty acid in Pug earwax samples was the lowest level among the breeds examined (Table 1). Moreover, *M. pachydermatis*-positive cases in the Pug (Table 2) were very few. On the other hand, the Shetland sheep dog and Siberian husky had a high incidence of otitis externa with *M. pachydermatis* in spite of being erect ear types, and these breeds had high fatty acid levels in the earwax. These results suggest that although the incidence of otitis externa in dogs relates to the ear pinna shapes, it depends preferably on the amounts of lipids secreted by the apocrine glands and the majority of *M. pachydermatis* preferred fatty acids for their habitat and growth followed by auditory inflammation.

Table 2. *Malassezia pachydermatis* isolation and total fatty acid levels in the cerumen of otitis cases

Breed	No. of cases	No. of cases positive for *M. pachyderamatis* growth	Average of total fatty acid level: μM
Pendulous ears			
Maltese	16	11	170.5
Labrador Retriever	12	7	158.0
Pug	9	2	55.4
Shih Tzu	8	5	125.8
Poodle	7	3	178.6
Beagle	5	1	148.2
Dachshund	4	3	225.8
Mixed	4	3	100.5
Cavalier King Charles Spaniel	1	1	(37.5)
Miniature Schnauzer	1	1	(0)
Total	67	37 : 55.2%	138.5*
Erect ears			
Mixed	8	5	37.6
Yorkshire Terrier	5	1	87.2
Shetland Sheep Dog	4	3	114.5
Japanese Shiba	4	2	40.3
Siberian Husky	3	1	331.7
West Highland White Terrier	2	1	17.5
Pomeranian	1	1	(10)
Japanese Kishu	1	1	(66)
Total	28	15 : 53.6%	91.4

a) Myristic, palmitic, margaric, linoleic, oleic and stearic acids were measured.
( ): The value for a single sample.
*: After dismissing extraordinary values in the Siberian Husky, the average value in the pendulous ear group was significantly ($P<0.01$) higher than that in the erect ear group.

M. pachydermatis has been described as readily growing yeasts on non-supplemented media such as Sabouraud's dextrose agar [6] unlike *M. furfur* that growth of which *in vitro* is dependent on lipid supplementation [1]. Nevertheless, the majority of *M. pachydermatis* strains isolated in the present experiment utilized fatty acids and grew quickly in the media supplemented with fatty acids, although the kinds of fatty acids utilized depended on the isolates. Bond and Anthony reported that some *M. pachydermatis* strains isolated from healthy Beagle dogs showed signs of enhanced growth in a lipid-supplemented medium [1]. Since many *M. pachydermatis* strains in field conditions may possibly be cultured on lipid-supplemented media, thereafter an application of Sabouraud's dextrose agar supplemented with lipid is recommended in the veterinary clinic. Moreover, for the treatment of canine otitis externa, methods to dissolve lipid in the auditory canal and remove it out should be made practicable. This kind of method is expected to be applied for the treatment of dermatitis with *M. pachydermatis*.

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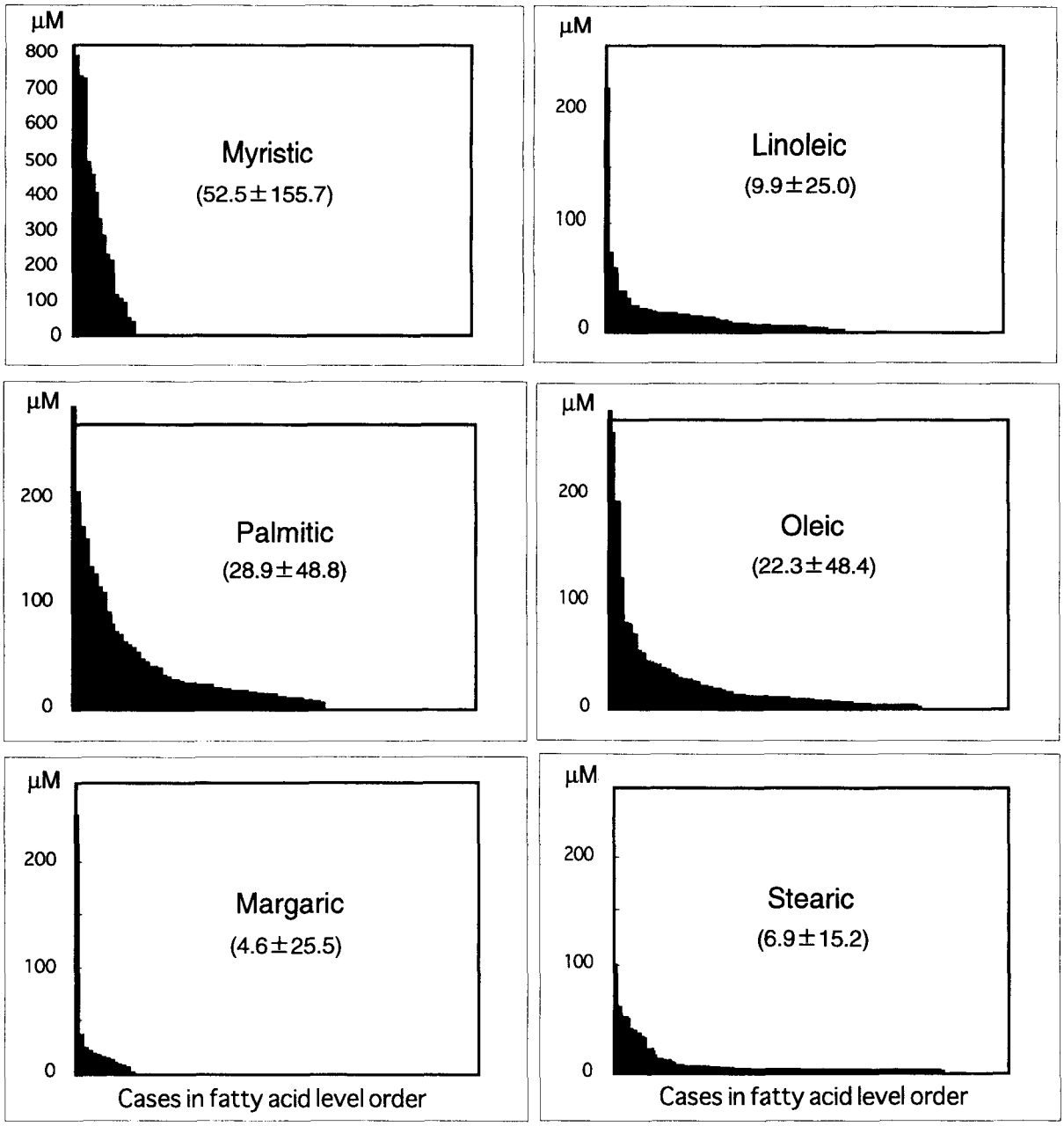

Fig. 1. Fatty acid levels (μM) in 95 ear cerumen specimens of canine otitis externa in level order. Myristic and margaric acids were detected in a limited number of cases, but the levels of myristic acid were very high. Other fatty acids were found in almost all specimens. Parentheses show the averages and standard deviations.

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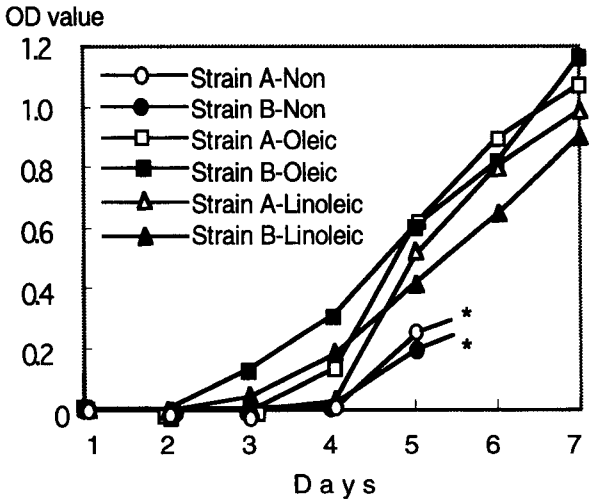

Fig. 2. Growth curves of *Malassezia pachydermatis* in Sabouraud's dextrose broth with fatty acids. This preliminary test was done on two representative strains with oleic and linoleic acids. Neither strain had clear growth without fatty acids even after 4 days' incubation. *: After 5 days, OD values for the strains without fatty acids could not be measured due to agglutination of the yeast cells.

Table 3. Utilization of fatty acids by *Malassezia pachydermatis*

Strains examined	Number of strains: More growth with fatty acids	Number of strains: Unrelated to fatty acids
42	31 (73.8)	11 (26.2)

( ): Percentage.

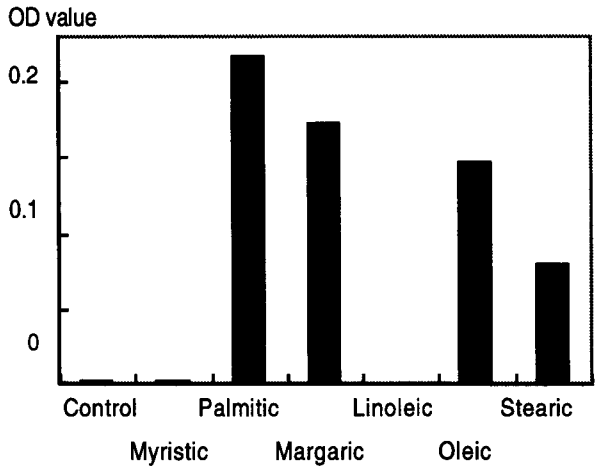

Fig. 3. Growth of *Malassezia pachydermatis* strain No. 4 as an example in Sabouraud's dextrose broth with each of 6 fatty acids. OD values after 3 days' incubation are shown.