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Original Article

Molecular Epidemiology of a Major Subgroup of *Arthroderma benhamiae* Isolated in Japan by Restriction Fragment Length Polymorphism Analysis of the Non-Transcribed Spacer Region of Ribosomal RNA Gene

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SUMMARY: *Arthroderma benhamiae* vectored by small animals, such as household pets, causes tinea lesions on human skin. The number of tinea cases caused by this species is increasing in Japan. We attempted to develop a simple molecular method for strain discrimination, which is expected to be useful in molecular epidemiology. Out of the 61 strains of *A. benhamiae* registered at our institute, 46 *A. benhamiae* strains showed very high degrees of sequence similarity on cluster analysis of the internal transcribed spacer regions of ribosomal RNA (rRNA) genes. These 46 strains, including 22 strains isolated from Japan, were further used for strain typing by analyzing the non-transcribed spacer (NTS) region of the rRNA gene. Polymerase chain reaction was performed using a primer pair designed for amplification of a part of the NTS region, and the amplicons were successfully discriminated by restriction fragment length polymorphism (RFLP) analysis performed using *Mva*I. RFLP analysis showed 11 NTS types (NTS1–NTS11) among the 46 strains. Out of the 22 Japanese strains, 10 were of the NTS8 type; 6, of the NTS1 type; 3, of the NTS2 type; and 3, of the NTS5 type. Molecular typing showed consistency of NTS types among the strains isolated from different lesions on the same patient, among the strains derived from the same family, and among the strains from pets and their owners. We observed that 3 out of the 4 NTS types among the Japanese strains were detected outside Japan as well.

INTRODUCTION

Dermatophytosis has the highest morbidity rate among skin diseases, and it occurrs in over 25 million patients among the 125 million population of Japan (1). The diagnosis of dermatophytosis is usually confirmed by performing a direct KOH examination and conventional fungal culture. However, sometimes, intraspecies differentiation is required to understand the transmission of dermatophytes between people and animals. On such occasions, sensitive techniques are required for strain identification or intraspecies typing of each dermatophyte.

Arthroderma benhamiae is one of the teleomorphic species of the *Trichophyton mentagrophytes* complex (2,3), which comprises the second most common causative agents of dermatophytosis. The incidence rates of dermatophytosis caused by *A. benhamiae* vary widely between humans and animals. In 1980, *A. benhamiae* isolates were not detected in an intensive mating study in Japan (4). However, in 1997, the first isolates of *A. benhamiae* were cultured from a rabbit in Japan (5). The strains isolated from a family with human dermatophytosis and their household pet (a rabbit) in 1996 were later identified as *A. benhamiae* strains (6). Several isolates of *A. benhamiae* have since been cultured from human and animal specimens in Japan, and the origins of these strains and the pathway of their dissemination are of epidemiological interest.

Ribosomal RNA (rRNA) genes are widely analyzed for taxonomic and clinical microbiological purposes. The internal transcribed spacer (ITS) regions of these genes are useful for species-level identification, and sometimes, intraspecies-level identification of dermatophytes. Indeed, restriction fragment length polymorphism (RFLP) analysis of ITS regions using *Mva*I and *Hin*fI is useful not only for the discrimination of *A. benhamiae* from other members of the *T. mentagrophytes* complex, but also for some intraspecies typing, such as detection of *A. benhamiae* var. *erinacei*, African race, and Americano-European race (7). However, analysis of the non-transcribed spacer (NTS) region of the rRNA genes has been reported to be a more robust technique for strain typing of some dermatophyte species, such as *Trichophyton rubrum* (8), *Trichophyton tonsurans* (9–12), and *T. mentagrophytes* var. *interdigitale* (13–15). Our previous study indicated that Southern blot hybridization of the NTS region is useful for the discrimination of *A. benhamiae* strains (16), and simple molecular methods are required to be developed for this purpose. Here, we report a simple molecular method involving RFLP performed after polymerase chain reaction (PCR) targeting the NTS region of *A. benhamiae* and discuss the molecular epidemiology of this species in Japan.

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Table 1. *Arthroderma benhamiae* strains used in the present study

No.	KMU			Geographic origin	Source (Reference)	ITS-RFLP	DDBJ accession no.	NTS-RFLP (*Mva*I)
1	3151	RV26678		Belgium	tester strain (3)	AE	AB686464	—
2	3152	RV26680		Belgium	tester strain (3)	AE	AF170457	—
3	3153	CDC X798a		USA	type culture (2)	AE	AB458216	—
4	3154	CDC X797A		USA	type culture (2)	AE	AB593394	—
5	3158	RV30000		Belgium	tester strain (3)	Af	AF170456	—
6	3159	RV30001		Belgium	tester strain (3)	Af	AB593395	—
7	4136			Shimane, Japan	human:tinea corporis (neck) (6)	MS	AB048192	NTS 1
8	4137			Shimane, Japan	rabbit (6)	MS	AB686465	NTS 1
9	4145	VUT97010		Hyogo, Japan	rabbit (5)	MS	AB686466	NTS 1
10	4146	VUT00001		Hyogo, Japan	guinea pig	MS	AB686467	NTS 2
11	4147	VUT00002		Saitama, Japan	rabbit	MS	AB686468	NTS 1
12	4148	VUT00003		Saitama, Japan	rabbit	MS	AB686479	NTS 8
13	4169			Gifu, Japan	human:tinea corporis (face) (19)	MS	AB088678	NTS 8
14	4170			Gifu, Japan	human:tinea manus (19)	MS	AB686470	NTS 8
15	4231			Nagasaki, Japan	human:tinea corporis (face) (21)	MS	AB686471	NTS 8
16	4232			Nagasaki, Japan	human:tinea corporis (scrotum) (21)	MS	AB686472	NTS 8
17	4233			Nagasaki, Japan	rabbit (21)	MS	AB686473	NTS 8
18	4234			Nagasaki, Japan	human:tinea corporis (face) (21)	MS	AB686474	NTS 8
19	4426			Tokyo, Japan	human:tinea capitis (kerion celsi) (20)	MS	AB686475	NTS 1
20	4443			Korea	human:tinea corporis (chest, abdomen)	Af	AB686476	—
21	4444			Korea	human:tinea corporis (face)	Af	AB686477	—
22	4445			Korea	human:tinea corporis (arm:Rt)	Af	AB686478	—
23	4446			Korea	human:tinea corporis (face)	Af	AB686779	—
24	4447			Korea	human:tinea corporis (face, chest)	Af	AB686780	—
25	4448			Korea	human:tinea corporis (arm:Lt)	MS	AB686781	NTS 8
26	4698			Yamagata, Japan	human (22)	MS	AB686782	NTS 8
27	4699			Yamagata, Japan	human (22)	MS	AB686783	NTS 8
28	4700			Yamagata, Japan	rabbit (22)	MS	AB686784	NTS 8
29	6079			Gifu, Japan	human:tinea unguium	MS	AB686785	NTS 5
30	6089			Gifu, Japan	human:tinea capitis (kerion celsi)	MS	AB686786	NTS 5
31	6113			Nagasaki, Japan	human:tinea corporis	MS	AB686787	NTS 1
32	6282			Tokyo, Japan	human:tinea corporis	MS	AB593396	NTS 2
33	6283			Tokyo, Japan	guinea pig	MS	AB686788	NTS 2
34	6365			Gifu, Japan	dog	MS	AB686789	NTS 5
35	6832	CBS806.72	RV14387ATCC28061	France	guinea pig	—	AB458214	—
36	6833	CBS807.72	RV14988ATCC28063	Spain	human	—	AB458213	—
37	6834	CBS808.72	RV27926ATCC28065	South Africa	human	Af	AB458212	—
38	6835	CBS809.72	RV28067ATCC28067	Belgium	dog	MS	AB686527	NTS 1
39	6836	CBS934.73		Germany		MS	AB458215	NTS 6
40	6837	CBS280.83		Netherlands	human:tinea corporis(hand)	MS	Z98016	NTS 1
41	6838	CBS112368		Switherland	human	MS	AB686490	NTS 7
42	6839	CBS112369		Switherland	human	MS	AB686491	NTS 7
43	6909			Ishikawa, Japan	human:tinea corporis (arm:Rt)	AE	AB678444	—
44	8033	IFM41159		Finland	guinea pig	MS	AB458148	NTS 2
45	8034	IFM41160		Finland	guinea pig	MS	AB458149	NTS 4
46	8035	IFM41161		Finland	guinea pig	MS	AB458150	NTS 9
47	8036	IFM41162		Finland	guinea pig	MS	AB458151	NTS 9
48	8037	IFM41166		Finland	guinea pig	MS	AB458155	NTS 11
49	8038	IFM41167		Finland	guinea pig	MS	AB458156	NTS 2
50	8039	IFM41168		Finland	guinea pig	MS	AB458157	NTS 2
51	8040	IFM41169		Finland	guinea pig	MS	AB458158	NTS 3
52	8041	IFM41170		Finland	guinea pig	MS	AB458159	NTS 3
53	8042	IFM41176		Finland	rabbit	MS	AB458165	NTS 4
54	8043	IFM41177		Finland	guinea pig	MS	AB458166	NTS 4
55	8044	IFM41178		Finland	guinea pig	MS	AB458167	NTS 11
56	8045	IFM41180		Finland	guinea pig	MS	AB458169	NTS 9
57	8046	IFM41181		Finland	guinea pig	MS	AB458170	NTS 9
58	8047	IFM41188		Finland	rabbit	MS	AB458177	NTS 10
59	8048	IFM41194		Finland	rabbit	MS	AB458183	NTS 10
60	8049	IFM41196		Finland	cat	MS	AB458185	NTS 7
61	8050	IFM41199		Finland	guinea pig	MS	AB458187	NTS 9

The strains were classified into the AE type, which belongs to the same cluster as the Americano-European race tester strains, and the Af type, which belongs to the same cluster as the African race tester strains. Five strains of the AE type, nine strains of the Af type, and one strain each of *A. benhamiae* var. *erinacei* and *A. benhamiae* var. *caviae* were obtained. Forty-six strains belonging to neither of the above types (i.e., major subgroup, MS) were subjected to the NTS analysis.

KMU, Department of Dermatology, Kanazawa Medical University, Ishikawa, Japan; RV, Collection R. Vanbreuseghem (Institute of Tropical Medicine, Antwerp, Belgium); CDC, Centers for Disease Control and Prevention, Atlanta, Ga., USA; VUT, Department of Veterinary Internal Medicine, Faculty of Agriculture, University of Tokyo, Tokyo, Japan; CBS, Centraalbureau voor Schimmelcultures, Baarn, Netherlands; ATCC, American Type Culture Collection, Manassas, Va., USA; IFM, Medical Mycology Research Center, Chiba University, Chiba, Japan; ITS-RFLP, restriction fragment length polymorphism in rRNA gene internal transcribed spacer region; DDBJ accession no., DNA Data Bank of Japan accession number; NTS-RFLP, restriction fragment length polymorphism in rRNA gene non-transcribed spacer region.

MATERIALS AND METHODS

Strains: For performing the standard mating tests, we used 61 strains of *A. benhamiae*, including 2 type strains and 4 tester strains that were provided by the Institute of Tropical Medicine (Antwerp, Belgium). Strains isolated from humans and animals and preserved at the Medical Mycology Research Center, Chiba University (Chiba, Japan) and the Centraalbureau voor Schimmelcultures (Baarn, Netherlands) were also included in this study. Of these, 23 strains were Japanese isolates. The details of the strains are listed in Table 1.

DNA extraction: DNA was extracted from colonies grown on Sabouraud's agar slant or plate by performing the modified mini-prep method reported by Makimura et al. (17). Briefly, a small amount of mycelial mat (wet weight, ~20 mg) was harvested and homogenized in 300 μL of lysis buffer (200 mM Tris-HCl [pH 7.5], 0.5% [w/v] sodium dodecylsulfate, 25 mM ethylenediaminetetraacetic acid, and 250 mM NaCl). The homogenate was heated at 100°C for 5 min, followed by addition of 150 μL of 3 M sodium acetate (pH 7.0). The homogenate was then stored at −20°C for 5 min. In order to obtain a DNA-containing precipitate, 450 μL of isopropanol was added to the supernatant of the homogenate. The precipitate was washed in 70% ethanol, dried, and then dissolved in 50 μL of distilled water. It could then be used as a DNA template.

Screening of the ITS regions: The ITS regions were amplified using the universal primers ITS-1 (5′-TCC GTAGGTGAACCTGCGG-3′) or ITS-5 (5′-GGAAG TAAAAGTCGTAACAAGG-3′) and ITS-4 (5′-TCCT CCGCTTATTGATATGC-3′) (18) and the DNA extracts obtained from the 61 strains of *A. benhamiae* as the templates. The products were purified using the NucleoSpin Extract II (Macherey-Nagel, Düren, Germany), sequenced using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kits (Applied Biosystems, Foster City, Calif., USA), and an ABI PRISM 310 Genetic Analyzer. The phylogenetic tree analysis was performed subsequently using *Arthroderma vanbreuseghemii* (RV27960) as an outgroup strain. The strains were classified into the AE type, which belongs to the same cluster as the Americano-European race tester strains, and the Af type, which belongs to the same cluster as the African race tester strains. We observed that 5 strains were of the AE type, 9 were of the Af type, and 1 strain each belonged to *A. benhamiae* var. *erinacei* and *A. benhamiae* var. *caviae*. The 46 strains belonging to neither the AE type nor the Af type formed the major subgroup (MS) (Fig. 1) and were subjected to further analyses. Out of the 23 Japanese strains, 22 belongs to the MS, but the remaining 1 strain (KMU6909) isolated from a tinea corporis infection in Japan was of the AE type.

Determination of the structure of the NTS regions: The nucleotide sequences of the entire NTS regions of 2 strains of *A. benhamiae* (RV30001 = KMU3159 and KMU6113) were determined for designing new primers for molecular typing. The RV30001 strain has been

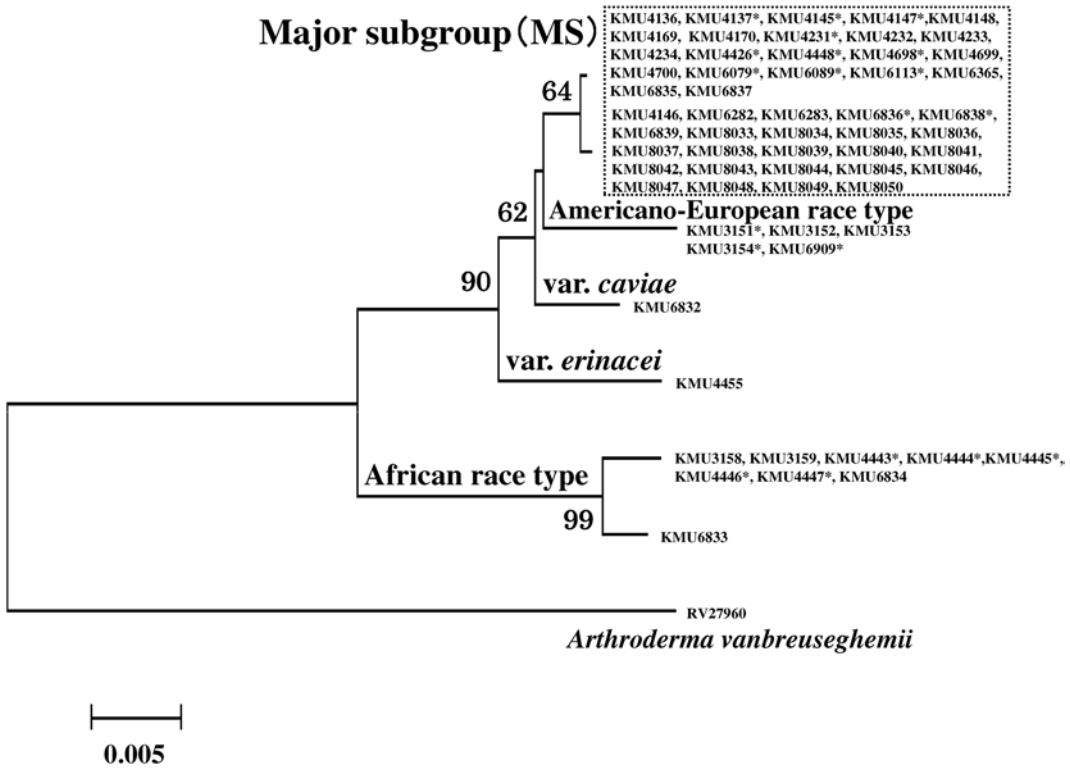

Fig. 1. Neighbor-joining tree based on sequences of the internal transcribed spacer regions of the *Arthroderma benhamiae* strains used. Forty-six strains, including 22 of 23 strains isolated in Japan, were clustered together. The cluster was apart from the clusters composed by the Americano-European race tester strains, the African race tester strains, and strains of var. *caviae* or var. *erinacei*. The 46 strains in the newly found cluster (major subgroup; MS) were subjected to further analyses. The sequence data of the strains marked with asterisks were obtained from GenBank. *Arthroderma vanbreuseghemii* (RV27960) was used as an outgroup.

widely used as an African race tester strain in mating tests. KMU6113 is a clinical isolate belonging to the MS in the tree of the present ITS analysis. The PCR primers were designed on the basis of the sequences of the NTS regions of *T. rubrum* (AB222887) and *T. tonsurans* (AB292646), registered in GenBank with the indicated accession numbers. The PCR products were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kits by the previously described method. We also determined the partial sequences of the NTS regions of KMU6282, KMU6089, and KMU4169, all of which were included in the MS.

PCR amplification of the NTS regions: On the basis of the comparison of the sequences of RV30001, KMU6113, and some other strains, a primer pair of AbNTS L896 (5′-TGGTGTACCATGGGCTAGAA-3′) and AbNTS R2425 (5′-ACTCGAAGGAGGCATAA GTC-3′) was designed to amplify the highly variable regions of the MS strains. The restriction enzyme *Mva*I (Takara Bio Inc., Otsu, Japan), which can help determine the differences between these strains, was selected on the basis of the results of a simulation study performed using the sequences of KMU6113, KMU6282, KMU6089, and KMU4169 with GENETYX MAC ver.10.1 (Software Development Co., Tokyo, Japan). The amplicons derived from these strains were digested with *Mva*I, and the predicted lengths of the digests corresponded with the actual bands on the gels.

Typing of 46 strains belonging to the MS: For typing the 46 strains that were determined to belong to the MS by sequence analysis of the ITS region, DNA fragments obtained by restriction enzyme digestion were prepared, and the digests were electrophoresed on 6% polyacrylamide gels, stained with ethidium bromide, and observed under an ultraviolet (UV) lamp.

RESULTS

Structure of the NTS regions (Fig. 2): The NTS regions of RV30001 and KMU6113 were 2325 bp (DDBJ accession no. AB 685332) and 3288 bp (DDBJ accession no. AB 685331) in length, respectively. The upper (i.e., 5′-end) half of the NTS region of RV30001 contained 2 repetitive units composed of the same sequences 210 bp in length. Repetitive units were not prominent in the lower (i.e., 3′-end) half of the NTS. The sequence of the upper half of the NTS region of KMU6113 was very different from that of the NTS region of RV30001 and contained 5 homologous units, i.e., 1 unit was 205 bp in length, 2 units were 219 bp in length, and 2 units were 233 bp in length. However, the lower half of the NTS region of KMU6113 was very similar to that of RV30001, and the lowermost 210 bp were completely identical in the 2 strains. We also determined the sequences of amplicons generated by the primer pair of AbNTS L896 and AbNTS R2425 from the following 4 MS strains: KUM6113, KMU6282, KMU6089, and KMU4169. We found variable numbers (from 2 to 5) of repetitive units composed of relatively similar sequences that were 205 bp to 233 bp in length, and these sequences were located between consensus structures that were 85 bp and 354 bp in length.

Typing of 46 strains belonging to the MS: The 46 strains classified as belonging to the MS because of their very similar ITS sequences, could be classified under 11 independent molecular types in the RFLP analysis of

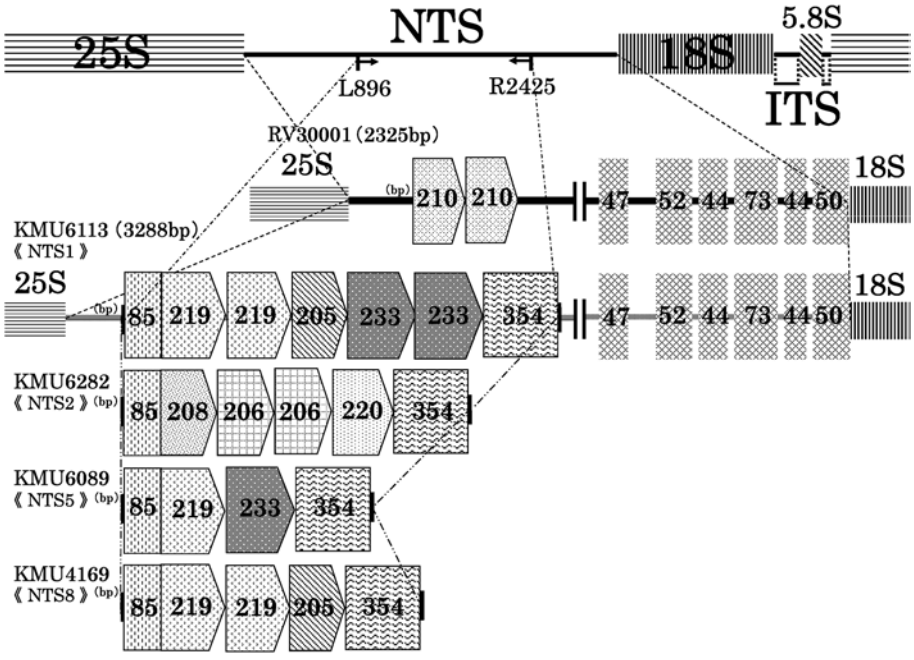

Fig. 2. Structures of the NTS region of the ribosomal RNA genes of *A. benhamiae*. The NTS regions of RV30001 (=KMU3159, African race) and KMU6113 (MS) were 2325 bp and 3288 bp in length, respectively. The 5′-end of the NTS of RV30001 contained 2 repetitive units composed of the same sequence 210 bp in length. The 5′-end of the NTS of KMU6113 was very different from that of RV30001, and contained 5 units between 205 bp and 233 bp in length. The sequences of the 3′-end of the 2 strains were very similar. Variable numbers (from 2 to 5) of repetitive units composed by relatively similar sequences 205 bp to 233 bp in length were located between consensus structures 85 bp and 354 bp in length in NTS1, 2, 5, and 8 strains.

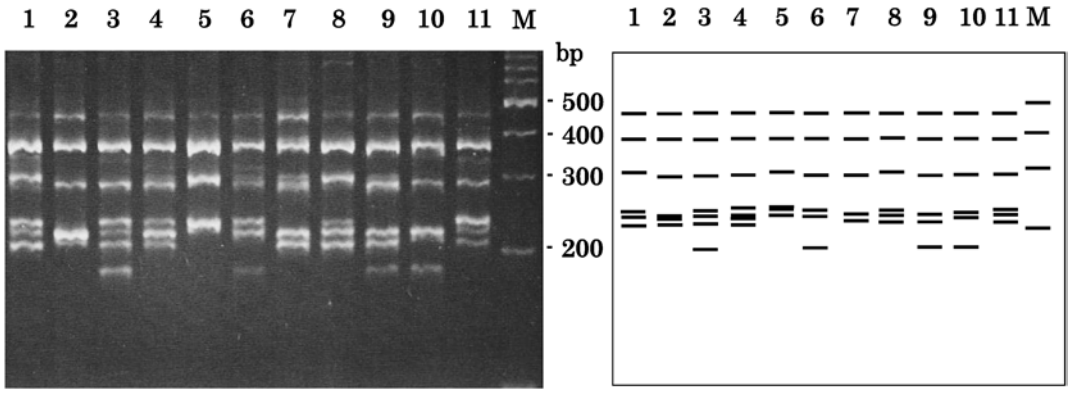

Fig. 3. RFLP patterns of the ribosomal RNA gene NTS region of the major subgroup of *A. benhamiae*. The NTS regions were amplified with the primers AbNTS L896 and AbNTS R2425, and the amplicons were then digested with *Mva*I. Lane 1, KMU6835 = NTS1; Lane 2, KMU8033 = NTS2; Lane 3, KMU8040 = NTS3; Lane 4, KMU8042 = NTS4; Lane 5, KMU6089 = NTS5; Lane 6, KMU6836 = NTS6; Lane 7, KMU6838 = NTS7; Lane 8, KMU4169 = NTS8; Lane 9, KMU8035 = NTS9; Lane 10, KMU8048 = NTS10; Lane 11, KMU8044 = NTS11; M, Molecular size marker. Right panel shows schematic illustrations of banding profiles.

amplicons generated using the primer pair of AbNTS 896 and AbNTS 2425; we named these types NTS1 to NTS11 (Fig. 3). Of the 46 strains, 11 were of the NTS8 type; 8, of the NTS1 type; 6, of the NTS2 type; 5, of the NTS9 type; 3 each, of the NTS4, NTS5, and NTS7 types; 2 each, of the NTS3, NTS10, and NTS11 types; and 1, of the NTS6 type. The present molecular epidemiological study showed consistency in the NTS types between the strains isolated from different lesions in the same patients, between the strains isolated from members of the same family, and between the strains isolated from pets and their owners (Table 1). For example, strains isolated from an owner (KMU4136) (6) and from her pet rabbit (KMU4137) (6) were of the NTS1 type, and 2 strains isolated from different lesions in 1 patient (KMU4169 and KMU4170) (19) were of the NTS8 type. Moreover, the NTS1, NTS2, and NTS8 strains were isolated from geographically distant areas of Japan (Fig. 4A): the NTS1 strains were from Hyogo (KMU4145, rabbit, 1998) (5), Saitama (KMU4147, rabbit, 2000), Tokyo (KMU4426, human case of tinea capitis, 2000) (20), and Nagasaki (KMU6113, human case of tinea corporis, 2007); the NTS2 strains were from Hyogo (KMU4146, 2000) and Tokyo (KMU6282, human case of tinea corporis; KMU6283, guinea pig, 2007); and the NTS8 strains were from Nagasaki (KMU4231–4234, pet rabbits and human cases of tinea corporis, 2001) (21) and Yamagata (KMU4698–4700, human cases of tinea corporis and pet rabbit, 2004) (22). The NTS1, NTS2, and NTS8 strains were also isolated from outside Japan: NTS1 strains, from Belgium and the Netherlands; NTS2 strains, from Finland; and NTS8 strains, from Korea (Fig. 4B). On the other hand, NTS5 strains (KMU6079, KMU6089, and KMU6365) were detected only in Gifu in Chubu (central part of the Honshu Island), Japan.

We also applied this method for analyzing the strains other than those belonging to the MS, but there were no strains showing the same banding profiles as those belonging to the 11 NTS types (data not shown).

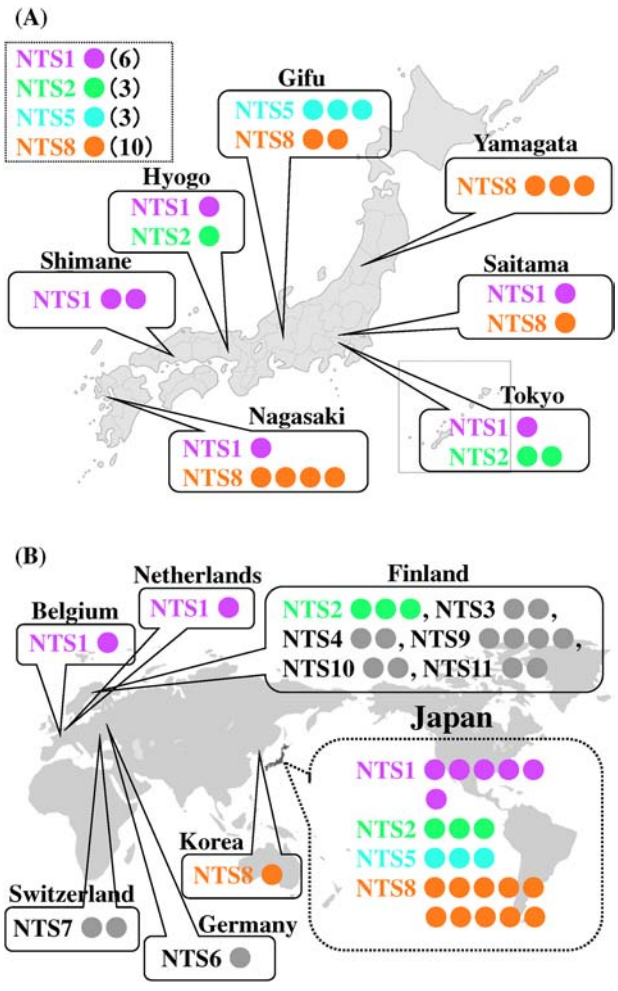

Fig. 4. (A) Distribution of the NTS subtypes of *A. benhamiae* in Japan. The 22 Japanese strains in the major subgroup were composed of NTS8 (n = 10), NTS1 (n = 6), NTS2 (n = 3), and NTS5 (n = 3). (B) Distribution of the NTS subtypes of *A. benhamiae* around the world. A total of 46 strains in the major subgroup were analyzed. Strains showing NTS1, 2, and 8 were found outside of Japan, but NTS5 were found only in Japan.

DISCUSSION

We collected 23 Japanese strains of *A. benhamiae*, including the first isolates (5,6) from 1996, some of which had been documented in the literature (19–26). These strains were speculated to have been imported from abroad after the survey in 1980 (4). Evaluation of the spread of the infection caused by the fungus by sequence analysis of ITS regions was insufficient. We found only 2 molecular types among the 23 strains isolated in Japan—22 strains belonged to the MS and 1 strain was of the AE type. Analyses of the NTS region are expected to lead to more precise intraspecies subtyping. Our previous study of this region using Southern blot hybridization identified 6 molecular types among 13 *A. benhamiae* strains isolated in Japan before 2001 (16). However, Southern blot hybridization has some disadvantages in that it requires technical skill, a considerable amount of total cellular DNA for the analysis, and very precise conditions for electrophoresis that are difficult to replicate; further, it is usually very difficult to compare the results from different laboratories, and the procedure is time consuming. Moreover, typing based on banding profiles is sometimes very difficult because of the existence of additional faint bands. In our previous study (16), the banding profiles of the strains isolated from an owner and her pet rabbit (KMU4136 and KMU4137) were very similar in appearance but were not completely identical because of the presence of additional faint bands, when we had expected to observe the same banding profile for the strains. On the other hand, banding profiles observed in the present study were clearer, and the discrimination power of this method was better than that of Southern blot hybridization. In addition, it took only 7 h to obtain the results, and this method was suitable for analyzing a number of strains. The present method facilitates the comparison of data from different laboratories and is suitable for molecular epidemiology. The limitation of this method is the very high specificity of the primer pair to the MS strains. We found poor production of amplicons from the strains of the Af type by the method used in this study (data not shown), which may have been due to the low affinity of the primer pair to the Af type strains. Thus, alteration of primer sequences and/or PCR conditions would be required to study the strains of the Af type by this method.

Our previous findings (16) and the results of the present study indicate that familial outbreaks of *A. benhamiae* infection still occur via household pets, such as rabbits and guinea pigs. Strains of the same subtypes, such as NTS1 and NTS8, were isolated from geographically distant areas of Japan, i.e., from Kyushu Island to Kanto (central-eastern part of Honshu Island) or Tohoku (northern part of Honshu Island). These data indicate that *A. benhamiae* spreads by transportation of animals contaminated with the species by animal breeders or pet shops. Our study also indicated that the species had been brought into Japan with imported animals on several occasions. Indeed, NTS1 strains have been found among the strains isolated in the Netherlands and Belgium, an NTS2 strain was isolated in Finland, and an NTS8 strain was isolated in Korea. These findings are consistent with the theory of import of the species from outside Japan. Between 2007 and 2008, 3 strains of the NTS5 type were independently isolated only from Gifu, and this molecular type has not been detected elsewhere to date. These findings suggest that NTS5 may be an imported subtype and not a domestic fungal flora found in Japan, because this subtype is not predominant in Japan, and its regional distribution is very restricted. However, this type is yet to be isolated from outside Japan.

The relationships between the molecular types of these strains and animal species or between the molecular types and pathogenesis to human skin are of additional epidemiological interest. *A. benhamiae* was first described as a teleomorph resulting from a mating test between 2 *T. mentagrophytes* strains isolated from a dog and a patient (2). However, these species are now mainly being isolated from rabbits and guinea pigs. In the MS strains, some molecular types appeared to be very species specific: strains of the NTS2, NTS3, NTS9, and NTS11 types were isolated only from guinea pigs, with an exceptional case of a strain isolated from a human patient. On the other hand, strains of the NTS1 and NTS8 types tended to be isolated from rabbits, strongly supporting the hypothesis that the source of tinea corporis was a pet-shop worker (19) infected by an NTS8 strain from the rabbits in the shop. NTS4 may be less species specific because these types were isolated from both rabbits and guinea pigs. The relationships between the pathogenesis in humans and molecular types are not yet clear. All the 7 cases of infection in humans caused by NTS8 strains were of tinea corporis, tinea manus, and tinea cruris, and the degrees of inflammation in the lesions were rather mild. Kerion celsi, the most inflammatory form of tinea capitis, was caused by 1 of 3 NTS1 strains and 1 of the 2 NTS5 strains, but the number of cases was too small to conclude that these subtypes are highly pathogenic.

In conclusion, we propose a simple PCR-based method that can be used to estimate the route and course of *A. benhamiae* infection. This method will be useful for molecular epidemiological studies on a number of fungal strains. In addition, since the results obtained by this method are stable, data from different laboratories can be compared better.

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Conflict of interest None to declare.

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