จีโนไทป์ของอะแคนทามีบาจากดินในจังหวัดนราธิวาส ประเทศไทย

Genotyping of *Acanthamoeba* spp. from Soil in Narathiwat Province, Thailand

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บทคัดย่อ

อะแคนทามีบาเป็นอะมีบาที่ดำรงชีวิตอย่างอิสระ พบได้ทั่วโลก บางจีโนไทป์ทำให้เกิดโรคในกระจกตาและระบบประสาทส่วนกลาง การศึกษาครั้งนี้ ได้นำอะแคนทามีบา (44 ไอโซเลท) ที่ได้จากการเพาะเลี้ยงและบ่งชี้ภายใต้กล้องจุลทรรศน์จากตัวอย่างดินใน 3 อำเภอ (บาเจาะ ยี่งอ และระแงะ) จังหวัดนราธิวาสจากการศึกษาก่อนหน้านี้ มาทำการยืนยันชนิดและจีโนไทป์ของอะแคนทามีบาโดยวิธี PCR และหาลำดับดีเอ็นเอ พบว่าเป็นอะแคนทามีบาร้อยละ 34.1 (15/44) ประกอบด้วยจีโนไทป์ T3 (3 ไอโซเลท) T4 (5 ไอโซเลท) T5 (4 ไอโซเลท) T17 (1 ไอโซเลท) และ T18 (2 ไอโซเลท) จีโนไทป์ T3, T4, T5 และ T18 มีรายงานว่ามีความเกี่ยวข้องกับการทำให้เกิดโรคในมนุษย์ รายงานนี้เป็นรายงานแรกที่พิสูจน์การปนเปื้อนของอะแคนทามีบาก่อโรคในแหล่งดินของจังหวัดนราธิวาส

คำสำคัญ: อะมีบาที่อยู่อย่างอิสระ อะแคนทามีบา ดิน นราธิวาส จีโนไทป์

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Abstract

Acanthamoeba spp. are free-living amoeba which are widely distributed worldwide. Some genotypes are known to cause diseases in corneal and central nervous system. In the present study, *Acanthamoeba* spp. (44 isolates) obtained by culture and identify under microscope from soil in 3 districts (Bacho, Yi-ngo, and Ra-ngae) of Narathiwat province from our earlier survey were used. The identification and genotyping of *Acanthamoeba* spp. were performed by PCR reactions and DNA sequencing. The results showed that 34.1% (15/44) were positive for *Acanthamoeba* spp. and belong to genotypes T3 (3 isolates), T4 (5 isolates), T5 (4 isolates), T17 (1 isolate) and T18 (2 isolates). Genotypes T3, T4, T5 and T18 have been reported to associate with human diseases. This is the first report proving the contamination of pathogenic *Acanthamoeba* spp. in soil from Narathiwat province.

Keywords: Free-living amoeba, *Acanthamoeba* spp., Soil, Narathiwat, Genotype

Introduction

Acanthamoeba spp. are free-living amoeba found in several locations, such as water, soil, air and various other sources including medical equipment as well as from clinical samples (Siddiqui & Khan, 2012; Bunsuwansakul et al., 2019; Koyun, Koloren, Karaman, Tsiami, & Karanis, 2020). *Acanthamoeba* spp. are the main causative agents of granulomatous amoebic encephalitis (GAE), amoebic keratitis (AK), and disseminated tissue infection in humans (Di Cave et al., 2014; Casaro, Walochnik, Kohsler, & Rott, 2015). In Thailand, several cases of infection caused by *Acanthamoeba* spp. have been reported, e.g., patients with *Acanthamoeba keratitis* (Wanachiwanawin, Booranapong, & Kosrirukvongs, 2012) and GAE (Sangruchi, Martinez, & Visvesvara, 1994).

On the basis of the 18S rRNA gene sequence, 22 genotypes (T1-T22) of *Acanthamoeba* spp. were established (Coronado-Velazquez et al., 2020). Almost all genotypes except T7, T16 and T17 have been proven to be involved in GAE and AK diseases (Tanveer et al., 2013; Adamska, 2015). In this study, we classify the genotypes of *Acanthamoeba* spp. collected from soil samples from 3 districts in Narathiwat Province. The pathogenic genotypes were also identified.

Objective

To identify and genotyping *Acanthamoeba* spp. in soil collected from 3 districts, Bacho, Yi-ngo, and Ra-ngae, Narathiwat province, Thailand, using PCR technique targeting 18S rRNA and gene sequencing.

Research methodology

***Acanthamoeba* spp.**

Acanthamoeba spp. (44 isolates) obtained from our previous survey (Hounkong, Sukchan, Sujiwattanarat, Dangmanee, & Sornying, 2020) were used. Briefly, they were culture and identify under microscope from 60 soil samples collected from 3 districts (Bacho, Yi-ngo, and Ra-ngae) of Narathiwat province.

Polymerase chain reaction identification of *Acanthamoeba* spp.

DNA of 44 *Acanthamoeba* spp. was extracted using a commercial QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The 18S rRNA gene was amplified using the genus-specific primers JDP1 (5-GGCCCAGATCGTTTACCGTGAA-3) and JDP2 (5-TCTCACAAGCTGCTAGGGAGTCA-3) targeting a 450-500 bp fragment as previously described (Schroeder et al., 2001). All PCRs were carried out in a total volume of 20 uL containing 1X TopTaq Master Mix Kit (Qiagen, Hilden, Germany), 10 pmoL of each primer, and 100-200 ng of DNA template. Amplification conditions were as follows: an initial denaturing phase at 94°C for 5 min and 40 repetitions at 94°C for 1 min, 62°C for 1 min and 72°C for 1 min with an elongation step of 5 min at 72°C in the last cycle. The PCR products were visualized by electrophoresis on 2% agarose gel and were then purified using the GenepHlow™ Gel/PCR Kit (DFH100, Geneaid, New Taipei, Taiwan). Purified PCR products were sequenced (Macrogen Inc., Seoul, Korea).

Phylogenetic and genotyping analysis

The consensus sequences were compared with other *Acanthamoeba* sequences in the GenBank database using the Basic Local Alignment Search Tool BLAST (search engine and aligned using ClustalW in the BioEdit package 7.2.5) (Hall, 1999). A phylogenetic tree of the isolated *Acanthamoeba* was constructed with Molecular Evolutionary Genetics Analysis version 6.0 (MEGA6) software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) using the Neighbour-

Joining distance (NJ) and Maximum Parsimony (MP) methods with a generation of 1000 bootstrapped replicates.

Results

From 44 *Acanthamoeba* spp. that were identified under microscope, 15 were positive for partial DNA fragments of the 18S rRNA gene of *Acanthamoeba* spp. (Table 1). The phylogenetic trees had similar topologies in both NJ and MP methods. Therefore, we presented only the NJ tree. The NJ tree (Figure 1) shows the evolutionary relationship of 5 isolates (R6P2, R6P2-1, R6P2-3, R12P2, and Y3P2) that belonged to genotype T4 and 4 isolates (R9P2, R14P2, Y5P1, and Y8P2) that belonged to genotype T5. Three isolates (R10P1-3, R10P1-4, and R10P1-5) were clustered into genotype T3, while the B20P12 isolate was clustered into genotype T17 and the other two (Y16P2 and Y17) belonged to genotype T18. The percent sequence identity between our sequences and each genotype group ranged from 96.2 to 100%. The 18S rRNA sequences were deposited in the GenBank database under accession numbers: MZ413901-MZ413915 (Table 2).

Table 1 *Acanthamoeba* spp. from soil in Narathiwat province, Thailand identified under microscope and PCR technique

Sampling area	Sample Total	Microscopic	PCR technique
Bacho District	20	13	1
Yi-ngo District	20	16	5
Ra-ngae District	20	15	9
Total	60	44	15

Table 2 Genotyping of *Acanthamoeba* spp. from soil in Narathiwat province, Thailand

Isolate	Sampling area	Genotype	GenBank accession number
B20P12	Bacho District	T17	MZ413901
Y3P2	Yi-ngo District	T4	MZ413902
Y5P1	Yi-ngo District	T5	MZ413903
Y8P2	Yi-ngo District	T5	MZ413904
Y16P2	Yi-ngo District	T18	MZ413905
Y17	Yi-ngo District	T18	MZ413906
R6P2	Ra-ngae District	T4	MZ413907
R6P2-1	Ra-ngae District	T4	MZ413908
R6P2-3	Ra-ngae District	T4	MZ413909
R9P2	Ra-ngae District	T5	MZ413910
R10P1-3	Ra-ngae District	T3	MZ413911
R10P1-4	Ra-ngae District	T3	MZ413912
R10P1-5	Ra-ngae District	T3	MZ413913
R12P2	Ra-ngae District	T4	MZ413914
R14P2	Ra-ngae District	T5	MZ413915
B20P12	Bacho District	T17	MZ413901
Y3P2	Yi-ngo District	T4	MZ413902
Y5P1	Yi-ngo District	T5	MZ413903

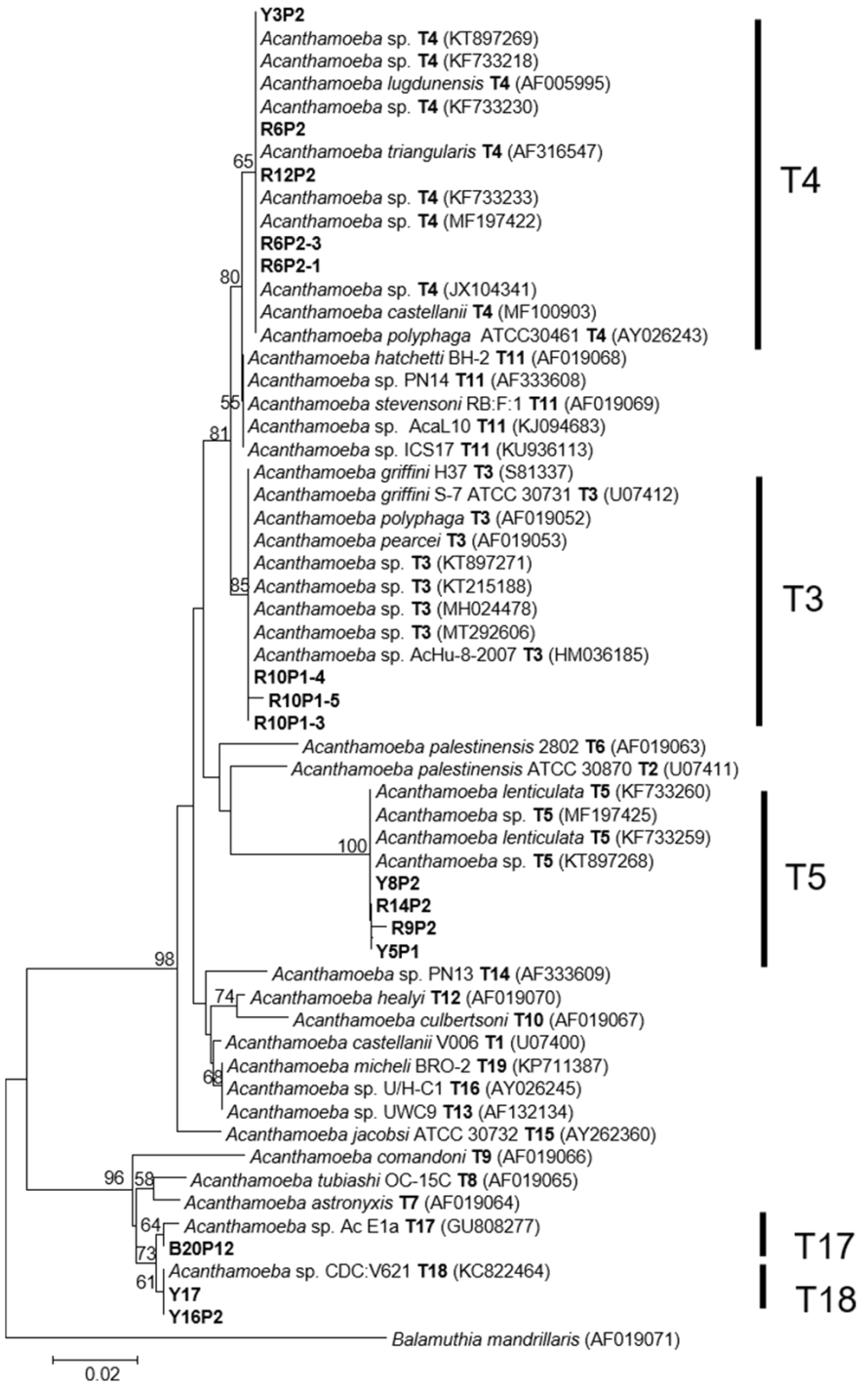

Figure 1 The phylogenetic relationships of 15 *Acanthamoeba* isolates inferred by Neighbor-Joining based on 18S rRNA gene sequence. *Balamuthia mandrillaris* was used as the root of the tree. Bootstrap values were inferred from 1000 replicates.

Discussion

Although *Acanthamoeba* spp. have been reported in soil, water, and dust samples (Nacapunchai, Kino, Ruangsitticha, Sriwichai, & Ishih 2001; Lekkla, Sutthikornchai, Bovornkitti, & Sukthana, 2005; Yaicharoen, Ngrenngarmlert, Thongmee, & Damsaman, 2007; Wannasan, Chaiwong, Bunchoo, & Morakote, 2009) , this study is the first to evaluate the genotypes of *Acanthamoeba* spp. in soil from Bacho, Yi-ngo and Ra-ngae district, Narathiwat province. Phylogenetic tree analysis identified genotypes T3 (3 isolates), T4 (5 isolates), T5 (4 isolates), T17 (1 isolate) and T18 (2 isolates) from selected soil samples. Of this, T4 genotype has the highest prevalence which comparable to others studies indicated the most common strain isolated from the environment worldwide including Thailand (Maciver, Asif, Simmen, & Lorenzo-Morales, 2013; Magnet et al., 2014; Thammaratana, Laummaunwai, & Boonmars, 2016; Esboei et al., 2020). T4 has been identified as the predominant genotype involved with keratitis (Gatti et al., 2010; Di Cave et al., 2014; Jercic et al., 2019) . Furthermore, T4 genotype of *A. castellanii* and T5 genotype of *A. lenticulate* can cause cornea and central nervous system disease in humans (Niyyati et al., 2010; Adamska, 2015; Walochnik, Scheikl, & Haller-Schober, 2015). In addition, *Acanthamoeba* spp. genotypes T3 and T5 have been isolated from an *Acanthamoeba* keratitis patient (Ledee et al., 2009; Zhao, Sun, Zhao, & Xie, 2010). Genotype T17 found in a previous study is supposed to be non-pathogenic *Acanthamoeba* spp. (Siddiqui & Khan, 2012) but T18 genotype has been found to cause GAE (Matsui et al., 2018). Our findings of *Acanthamoeba* spp. genotypes T3, T4, T5 and T18 are serve as additional proof for the presence of pathogenic *Acanthamoeba* spp. in the area related directly to human activities.

Conclusion

The present study has contributed to molecular identifying *Acanthamoeba* spp. genotypes from soil in 3 districts, Bacho, Yi-ngo, and Ra-ngae, Narathiwat province, Thailand. The results have shown that T3, T4 and T5 are highly distributed in the samples. These pathogenic strains can be considered as important risk for possible sources of infection caused by *Acanthamoeba* spp.

Suggestion

Further research is required to determine the correlation between *Acanthamoeba* strains and clinical cases in the area.

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