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ORIGINAL ARTICLE

Evaluation of Expression of Lipases and Phospholipases of *Malassezia restricta* in Patients with Seborrheic Dermatitis

Yang Won Lee, Shin Yung Lee[1], Younghoon Lee[2], Won Hee Jung[1]

Department of Dermatology, School of Medicine, Konkuk University, Seoul,
[1]Department of Systems Biotechnology, Chung-Ang University, Anseong, [2]Department of Chemistry, KAIST, Daejeon, Korea

Background: *Malassezia* species (spp.) are cutaneous opportunistic pathogens and associated with various dermatological diseases including seborrheic dermatitis, dandruff and atopic dermatitis. Almost all *Malassezia* spp. are obligatorily lipid-dependent, which might be caused by lack of the myristic acid synthesis. Recent genome analysis of *M. restricta* and *M. globosa* suggested that the absence of a gene encoding fatty acid synthesis might be compensated by abundant genes encoding hydrolases, which produce fatty acids, and that lipases and phospholipases may play a role in virulence of the fungus. **Objective:** The current study aimed to investigate the contribution of lipases and phospholipases in virulence of the *M. restricta* as being the most frequently isolated *Malassezia* spp. from the human skin. **Methods:** Swap samples of two different body sites of at least 18 patients with seborrheic dermatitis were obtained and *in vivo* expression of lipases and phospholipases of *M. restricta* was analyzed by the gene specific two-step nested RT-PCR. **Results:** The results of the current study suggest that majority of the patients display expression of lipase RES_0242. **Conclusion:** These data imply a possible role of lipase in the host environment to produce free fatty acids for the fungus. **(Ann Dermatol 25(3) 310～314, 2013)**

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Corresponding author: Won Hee Jung, Department of Systems Biotechnology, Chung-Ang University, 4726 Seodong-daero, Daedeok-myeon, Anseong 456-756, Korea. Tel: 82-31-670-3068, Fax: 82-31-675-1381, E-mail: whjung@cau.ac.kr

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INTRODUCTION

Basidiomycete yeasts *Malassezia* species (spp.) are cutaneous microflora and are considered as opportunistic pathogens. *Malassezia* are associated with various dermatological diseases including seborrheic dermatitis, dandruff and atopic dermatitis[1,2]. Among *Malassezia* spp., *M. globosa* and *M. restricta* were most frequently isolated from patients with dermatitis. Furthermore, *M. restricta* was known to be the predominant species among Korean teens and young adults[3]. Almost all *Malassezia* spp. are obligatorily lipid-dependent, which might be caused by lack of the myristic acid synthesis－the precursor of long chain fatty acids. The cell wall of *Malassezia* spp. contains significantly higher lipid contents than non-pathogenic fungi, such as *Saccharomyces cerevisiae*, implying that lipid-dependency of the fungus plays a role in virulence[4]. Recent genome analysis of *M. globosa* suggested that the absence of a gene encoding fatty acid synthase might be compensated by abundant genes encoding hydrolases that produce fatty acids. The genome of *M. globosa* possesses 14 lipases and 9 phospholipases, and the study showed that many of them were expressed on human scalp to use host lipids[1]. These results led us to investigate contributions of lipases and phospholipases in virulence of *M. restricta* as being the most frequently isolated *Malassezia* spp. within the Korean population. We searched the unannotated and incomplete *M. restricta* genome for a lipase and a phospholipase with *M. globosa* sequences and

found three and one homologs respectively. The sequences of lipase and phospholipase homologs were used to design *M. restricta* specific primers for the current study. To investigate the expression of three lipases and a phospholipase of *M. restricta*, swap samples of two different body sites of at least 18 patients with seborrheic dermatitis were obtained, and the total RNA was directly extracted. Reverse transcription-polymerase chain reaction (RT-PCR) was performed and nested polymerase chain reaction (PCR) was carried out using *M. restricta* specific primers for lipases and phospholipases. Our results indicated that *M. restricta* was presented in body sites of patients and suggested that majority of the patients display expression of lipase RES_0242.

MATERIALS AND METHODS

Preparation of swap samples

Swap samples of two different body sites, forehead and cheek, from 18 patients with seborrheic dermatitis were obtained as described elsewhere[5]. Briefly, a rayon swap was rubbed multiple times across the body site selected. A swap was immediately placed in 1 ml Trizol (Life Technologies, Carlsbad, CA, USA) and snap- frozen in liquid nitrogen. Samples were melted after all swaps were collected and vortexed. Swaps were removed, and 0.2 g of glass beads was added. Samples were vortexed again and RNA was isolated as instructed by the manufacturer. All samples were treated with RNase-free DNase to eliminate possible genomic DNA contaminations. This study was approved by the Institutional Review Board of Konkuk University Hospital (KUH1120026).

Reverse transcription-polymerase chain reaction

Two-step RT-PCR using nested primers were used for the current study. cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Fermentas, Vilnis, Lithuania) and used as a template for the first round PCR. One micro-liter of the product of the first round PCR was used as template for the second round PCR using nested primers. Total 10 μl of the second round PCR was loaded on the 1.0% agarose gel, separated with electrophoresis and visualized. Gene specific primers used in the current study were listed in Table 1.

RESULTS

The recent genome sequencing data revealed that *M. globosa* possesses a total of 14 lipases and nine phospholipases. Among them, four lipases (MGL_3878, MGL_3507,

Table 1. Primers used in this study

Target gene	Name	Sequence	Homolog in *Malassezia globosa*
Primers for the first round PCR			
Actin	MRE.ACT.F	AGATCTGGCACCACACGTTCTAC	MGL_1986
	MRE.ACT.R	ACAGCTTCTCTTTAATGTCACGCAC	
Lipase MRE_3144	MRE.3144.F	TCGCATTATCCTGAGGGCACAAGG	MGL_0797
	MRE.3144.R	TTGAGTGTGGAAGTACACGCCCTG	
Lipase MRE_0571	MRE.0571.F	GTCGCGCATGTACTGGACAATG	MGL_3507
	MRE.0571.R	ACTGTACACGCTCAACTCGCTC	
Lipase MRE_0242	MRE.0242.F	GACCCTATCCTGACCTTTGACGAC	MGL_3878
	MRE.0242.R	TGGCTGGATTGGCATACACTCTCC	
Phospholipase MRE_3947	MRE.3947.F	CCCCAGGTATCGTACATCATTACGC	MGL_3326
	MRE.3947.R	ACGGACAAGAGGTCACTCATATGC	
Nested primers to the second round PCR			
Actin	MRE.ACTnes.F	CCTTCTCACTGAGGCCCCTCTGAAC	MGL_1986
	MRE.ACTnes.R	CTTGACAAGATGCTCTGTGAGGTC	
Lipase MRE_3144	MRE.3144nes.F	GCATGGCTTCCAGGAGGCCTAC	MGL_0797
	MRE.3144nes.R	GTGGTCGTAGAAGTTAGGGGCG	
Lipase MRE_0571	MRE.0571nes.F	GTTCAGCAGGTACGTGGTGACG	MGL_3507
	MRE.0571nes.R	GTAAGCCTACTTCGTCGCTGGTGG	
Lipase MRE_0242	MRE.0242nes.F	CCACGGCATCTACTTCCACACC	MGL_3878
	MRE.0242nes.R	CATTGTGCTCTATGGTATCACTTCAG	
Phospholipase MRE_3947	MRE.3947nes.F	GCTGTCGGAGCACCCTCCTTAC	MGL_3326
	MRE.3947nes.R	CTCCTTGCTCGTCCAGTTCTTGC	

PCR: polymerase chain reaction.

MGL_0799, and MGL_0798) and two phospholipases (MGL_4252 and MGL_3326) were expressed on human scalps[6]. We used sequences of *M. globosa* lipases and phospholipases to search homologous genes in the *M. restricta* genome and identified three lipases and a phospholipase. Gene specific primers and nested primers were designed using sequences of these homologs (Table 1). Total of 18 patients, ten females and eight males with ages from 26 to 80 years old, with seborrheic dermatitis, were chosen to investigate whether the expression of *M. restricta* lipases or the phospholipases was detected on their body sites. From the 18 patients, we were able to obtain a total of 29 swap samples 12 forehead and 12 cheek swap samples from 12 patients, two forehead swap samples from two patients, and three cheek swap samples from three patients. Total RNA was extracted from the swap samples and was subsequently used as a template for cDNA synthesis. Two separate PCR, which was named as two-step nested RT-PCR in the current study, were carried out to detect expression of lipase or phospholipase homologs in *M. restricta*. Synthesized cDNA and gene specific primers were used for the first round PCR, and nested primers for each lipase or phospholipase homolog and products from the first round PCR were used for the second round PCR.

Using the two-step nested RT-PCR analysis, we were able to detect expression of the *M. restricta ACT1* gene from the all swap samples except the cheeks of the patient 17. This result proved that *M. restricta* resides on the body sites of the patients tested in the current study. We also analyzed expression of lipases and phospholipases and found that 12 swap samples displayed expression of lipase RES_0242. Furthermore, our data showed that lipase RES_0571 was only expressed on the forehead of patient 13, and lipase RES_3114 was expressed on the forehead of patient 4 and on the cheeks of patient 7 and 10. A gene encoding phospholipase homolog RES_3947 was expressed on six swap samples (Fig. 1). Expression of the same lipases and phospholipases were also evaluated in the cells cultured *in vitro*. We found that lipase RES_0242 and lipase RES_3114 were not expressed *in vitro* and the results were summarized in Fig. 2. Thus, our data showed that majority of the patients displayed expression of lipase RES_0242 and suggested that the gene may play a significant role in the host environment such as contributing fatty acids production for *M. restricta*. Although it occurred from only three samples, lipase RES_3114 might also contribute to virulence since the gene was only expressed *in vivo*. Roles of phospholipase homolog RES_3947 and lipase RES_0571 were unclear because they were expressed *in vitro*.

DISCUSSION

Malassezia are lipophillic yeast and the recent genome sequencing analysis revealed that a fatty acid synthase gene is absent in the organisms. To compensate lipid dependency, *Malassezia* possess multiple lipases and phospholipases to generate lipids from the host. Among the 23 lipases and phospholipases identified from the genome of *M. globosa*, four lipases and two phospholipases were expressed on surface of the host[6]. These include lipase MGL_3057, lipase MGL_3878, lipase MGL_0799, lipase MGL_0798, phospholipase MGL_4252 and phospholipase MGL_3326. Lipase MGL_0799 was of particular interest because another independent study also identified it and indicated that the gene, designated as *LIP1*, is expressed on human skin. Although the whole genome sequence of *M. globosa* has been revealed and few studies carried out to analyze expression of lipases and phospholipases of the fungus, no information is currently available for *M. restricta*[5,7-10]. The recent study found that the most commonly identified *Malassezia* spp. is *M. restricta* rather than *M. globosa* in the seborrheic dermatitis patients—*M. restricta* was identified in 47.5% and *M. globosa* in 27.5%[11].

In this study, we identified genes encoding lipases and phospholipases of *M. restricta* and investigated whether

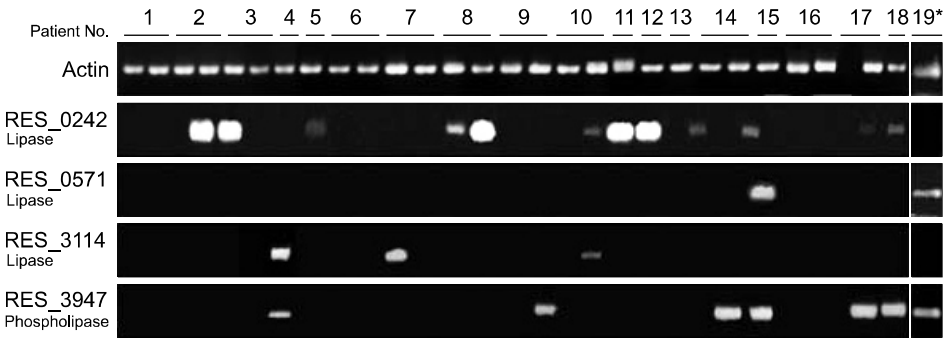

Fig. 1. Results from two-step nested polymerase chain reaction. *Malassezia restricta* was isolated from almost all patients except from the cheek of the patient 17. Expression of genes encoding lipase RES_0242 was most frequently detected across the samples. Lipase RES_0242 and lipase RES_3114 were only expressed *in vivo*. Asterisk indicates expression of the lipases and phospholipases in the cells cultured *in vitro*.

Patient No.	Sex	Age	Body site	RES_ACT	RES_0242	RES_0571	RES_3114	RES_3947
					Lipase	Lipase	Lipase	Phospholipase
1	F	26	Forehead					
			Cheek					
2	F	27	Forehead					
		27	Cheek					
3	F	27	Cheek					
		27	Forehead					
4	F	28	Forehead					
5	F	28	Cheek					
6	F	32	Forehead					
		32	Cheek					
7	F	38	Cheek					
		38	Forehead					
8	F	50	Forehead					
		50	Cheek					
9	F	54	Cheek					
		54	Forehead					
10	F	57	Forehead					
		57	Cheek					
11	M	22	Cheek					
12	M	27	Cheek					
13	M	28	Forehead					
14	M	36	Forehead					
		36	Cheek					
15	M	38	Forehead					
16	M	45	Cheek					
		45	Forehead					
17	M	62	Cheek					
		62	Forehead					
18	M	80	Forehead					
19*	*In vitro*							
Malassezia globosa hemolog				Actin	MGL_3878	MGL_3507	MGL_0797	MGL_3326

Fig. 2. Summary of the results of two-step nested polymerase chain reaction. Asterisk indicates expression of the lipases and phospholipases in the cells cultured *in vitro*. F: female, M: male.

those genes are expressed on the body sites of patients with seborrheic dermatitis. Results of our study revealed that *M. restricta* possesses homologs of at least four lipases and a phospholipase of *M. globosa*, and they were expressed in some of swap samples obtained from the patients. Specifically, we found that lipase RES_0242 was detected most frequently in the swap samples while its expression was not detected *in vitro*. We should note that *M. restricta* lipase RES_0242 was the closest homolog of *M. globosa* Lip1, of which homolog was also identified in *M. furfur*[7]. The Lip1 protein belongs to triglyceride lipase (EC 3.1.1.3), which hydrolyzes the ester bond of triglycerides to release free fatty acids and is considered as a class 3 lipase. The protein has also been considered as a virulence factor in other pathogenic bacteria and fungi[12]. Lipase RES_3114 might also be interesting. Although only in three cases, our data suggested that lipase RES_3114 is regulated similarly to lipase RES_0242—it was only expressed *in vivo*. These data imply a possible role of lipase RES_0242, and, perhaps, lipase RES_3114 in the host environment to produce free fatty acids for the fungus. We, however, did not observe any correlation between expression of genes encoding lipases and phospholipases, and age, gender and a body site. Therefore, future study requires increased number of samples and more sensitive detection methods to evaluate expression of the genes.

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