pH-Dependent Expression, Stability, and Activity of Malassezia restricta MrLip5 Lipase

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Background: The lipophilic yeasts Malassezia spp. are normally resident on the surface of the human body, and often associated with various skin diseases. Of the 18 known Malassezia spp., Malassezia restricta is the most predominantly identified Malassezia sp. found on the human skin. Malassezia possesses a large number of genes encoding lipases to degrade human sebum triglycerides into fatty acids, which are required not only for their growth, but also trigger skin diseases. Previously, we have shown that MrLIP5 (MRET_0930), one of the 12 lipase genes in the genome of M. restricta, and is the most frequently expressed lipase gene in the scalp of patients with dandruff.

Objective: In this study, we aimed to analyze the activity, stability, and expression of MrLip5, with particular focus on pH.

Methods: We heterologously expressed MrLip5 in Escherichia coli, and purified and analyzed its activity and expression under different pH conditions.

Results: We found that MrLip5 was most active and stable and highly expressed under alkaline conditions, which is similar to that of the diseased skin surface.

Conclusion: Our results suggest that the activity and expression of MrLip5 are pH-dependent, and that this lipase may play an essential role at the M. restricta-host interface during disease progression.

INTRODUCTION

Malassezia is the most abundant fungal genus isolated from human skin and is associated with various skin disorders including seborrheic dermatitis, dandruff, atopic dermatitis, and pityriasis versicolor1-5. Of the 18 currently identified Malassezia spp., Malassezia restricta is the most predominant species found on human skin1,2,6-8. Furthermore, the clinical significance of M. restricta in specific skin diseases has been suggested by numerous studies. Clavaud et al.2 and Park et al.9 quantitatively analyzed and compared M. restricta populations on the scalp of patients with dandruff and healthy individuals using a culture-independent method, and showed a significantly higher population of the species on the scalp of the former than that on healthy scalps. Additionally, Kato et al.10 quantified Malassezia spp.-specific immunoglobulin E (IgE) antibodies in sera from patients with atopic dermatitis and detected higher levels of M. restricta-specific IgE than those of other species.

One of the most notable physiological characteristics of Malassezia spp. is their lipid-dependency. Recent genome sequencing analysis has revealed that the lipophilic nature of Malassezia spp. is caused by the lack of a fatty acid synthase gene11-15. This physiological characteristic might be compensated for by secretion of lipolytic enzymes, including lipases, which act on carboxylic ester bonds, resulting in the production of free fatty acids16. Indeed, Malassezia possesses a large number of genes en-
coding lipases compared with other fungi\textsuperscript{11}, and secretes lipases to degrade triglycerides of human sebum into fatty acids, consuming the resultant metabolites for growth\textsuperscript{17-19}.

Moreover, a number of studies have suggested that the abnormal increase in unsaturated fatty acid levels, in particular oleic acid, generated by Malassezia lipases after consuming specific saturated fatty acids on the scalp surface, might be an etiological factor for dandruff, implying that such enzymes not only contribute to the survival of Malassezia on the host skin surface, but may also trigger skin disorders\textsuperscript{19,20}.

Extracellular lipases are also considered to be an important virulence factor of other pathogenic fungi. In particular, lipases of the human fungal pathogen Candida albicans are known to contribute to the successful colonization by fungal cells of host tissue during infection, as well as inducing morphological transitions of the fungal cells\textsuperscript{21,22}. To date, a total of 10 lipase genes (CaLIP1–CaLIP10) have been identified in C. albicans, and among these, CaLIP5, CaLIP6, CaLIP8, and CaLIP9 are expressed during infection in mice. Furthermore, a strain lacking CaLIP8 is significantly less virulent, suggesting the roles of these genes in pathogenesis\textsuperscript{23,24}. In the plant fungal pathogen Fusarium graminearum, disruption of FGL1, which encodes a lipase, results in reduction of fungal virulence in wheat and maize, further supporting the importance of lipases in pathogenic fungi\textsuperscript{25}.

Several studies have attempted to identify and biochemically characterize lipases in Malassezia spp.\textsuperscript{17,18,26-31} However, these studies were mainly focused on Malassezia spp. other than M. restricta, and no study has been carried out to understand the lipases of the most predominant Malassezia sp. on human skin. In a previous study, we sequenced the genome of M. restricta KCTC 27527, which was clinically isolated from a patient with dandruff, and identified 12 lipase genes, including MrLIP1\textsuperscript{4,17,30}. Moreover, we demonstrated that MRET_0930 (named MrLIP5), which encodes a lipase of the LIP family (PF03583), is the most frequently expressed lipase gene on the scalp of patients with dandruff, suggesting that MrLip5 may play a major role in M. restricta-host skin interactions\textsuperscript{14}. In the current study, we aimed to analyze the expression and activity of MrLip5 under different environmental conditions such as pH and temperature, which are the main factors that influence skin health.

**MATERIALS AND METHODS**

**Culture conditions**

The clinically isolated M. restricta strains KCTC 27524, KCTC 27527, KCTC 27529, KCTC 27539, and KCTC 27540, KCTC 27543, and KCTC 27550 were grown in Leeming and Notman\textsuperscript{32} agar medium (LNA; 0.5% [w/v] glucose, 0.01% [w/v] yeast extract, 1% [w/v] polypeptone, 0.8% [w/v] bile salt, 0.05% [w/v] glycerol monostearate, 0.05% [v/v] Tween\textsuperscript{®} 60 [Sigma, St. Louis, MO, USA], 0.1% [v/v] glycerol, 1.2% [w/v] agar and 0.5% [v/v] whole-fat cow milk) and cultured at 34°C for 3 days. Escherichia coli BL21 was grown in Luria-Bertani (LB) broth at 10°C or 37°C\textsuperscript{30}.

**Heterologous expression and purification of MrLip5**

To biochemically characterize MrLip5, we first amplified the coding region of the MrLIP5 gene, using the primers OPINM.MRET_0930.F (5’-AAGTCTCGTTCAGGGCCGGGTTCCATTACCTCAAGACGATCC-3’) and OPINM.MRET_0930.R (5’-ATGGCTCAAGAGCGTTTAATGTGCTTGCGCGTGCTTCTTC-3’), and the cDNA of M. restricta KCTC 27527 as template. The amplified PCR product was ligated into the pKpln and HindIII sites of plasmid pOPINM, which encodes 6× histidine (His) residues and maltose-binding protein (MBP) domain tags, using the In-Fusion\textsuperscript{®} HD Cloning Kit (Clontech Laboratories, Mountain View, CA, USA)\textsuperscript{33}. The constructed plasmid was designated pMJ002 and transformed into E. coli BL21. To express MrLip5, bacterial cells were cultured at 10°C overnight in the presence of 0.5 mM isopropyl β-D-1-thiogalactopyranoside, and the recombinant MrLip5 protein was purified using His GraviTrap\textsuperscript{TM} columns (GE Healthcare Life Science, Buckinghamshire, UK).

**Enzyme activity assays**

Lipase activity was evaluated spectrophotometrically using p-nitrophenylpalmitate, as described previously\textsuperscript{34}. For determination of the optimal pH for the lipase reaction, sodium citrate buffer (pH 3.0–6.0) or potassium phosphate buffer (pH 6.0–8.0) was used as a reaction buffer in the assay mixture (100 mM buffer, 0.5 mM p-nitrophenylpalmitate, 0.5% [v/v] Triton X-100 and 1 μg of the purified protein). The enzyme reaction was carried out at 37°C for 1 hour and arrested by the addition of two volumes of 1 M Tris buffer (pH 8.0). The optical absorption at 405 nm was measured to determine activity. To analyze stability, the enzyme was incubated over a range of different pH levels at 37°C for 12 hours, and enzyme activity was determined as described above\textsuperscript{35,36}.

**Generation of MrLip5 antiserum**

An antiserum against M. restricta MrLip5 was generated by a custom antibody production service using the synthesized peptide representing the protein (\textsuperscript{37}KGDISPGECDGHTKES\textsuperscript{38}, Young In Frontier Inc., Seoul, Korea). Briefly, for the first immunization, an emulsion produced by mixing
the antigen with complete Freund’s adjuvant was injected into various parts of rabbits. For the secondary immunization, a mixture of the antigen and incomplete Freund’s adjuvant was injected into rabbits four weeks later. A third immunization was performed using the same methods of the secondary immunization. A week later, rabbit blood was collected by heart puncture and antiserum against MrLip5 was purified.

**Western blot analysis**

To analyze the expression of MrLip5 under different pH and temperature conditions, *M. restricta* strain KCTC 27527 cells were cultured on LNA medium at pH 5.0, 6.0, 7.0, 8.0, and 9.0 at 34°C, or at pH 5.0, 7.0, and 8.0 at 30°C, 34°C, and 37°C for 3 days. Cells were harvested and suspended in cell lysis buffer (50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-KOH [pH 7.0], 140 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 0.1% [w/v] Na-deoxycholate, 1% [v/v] Triton X-100 and 1 mM phenylmethylsulfonyl fluoride). Whole-cell proteins from the cell suspension were extracted as described previously. Samples of 15 or 20 μg of whole-cell protein were loaded onto gels for western blotting of MrLip5, and western blot analysis was performed with MrLip5-specific antiserum (1 in 500 dilution) as a primary antibody and a goat anti-rabbit IgG-horseradish peroxidase was employed as the secondary antibody.

**RESULTS**

**Heterologous expression and purification of MrLip5**

Our previous genome sequencing analysis of *M. restricta* KCTC 27527 revealed that MRET_0930 has an open reading frame of 1,530 bp encoding a lipase family (PF03583) lipase MrLip5, which consists of 509 amino acids with a predicted molecular mass of 57.3 kDa (Fig. 1). To analyze the activity of MrLip5, the coding region of the MrLIP5 gene was cloned into the pOPINM plasmid (containing 6×His and MBP tags), and the recombinant protein was overexpressed in *E. coli* and purified (see MATERIALS AND METHODS). We attempted to remove MBP from purified MrLip5 but were unsuccessful; therefore, we transformed the same *E. coli* strain with the empty pOPINM plasmid without any insert, purified the MBP, and used it as a negative control throughout the study. After protein purification, we verified that the molecular weights of purified MBP and MrLip5-MBP were 42.5 and 68.5 kDa, respectively.

**Fig. 1.** Sequence alignment of MrLip5 and homologs in other fungi. MGL_3507 (XP_001729472.1), Malassezia globosa homolog; MSY001_2988 (XP_018741488.1), Malassezia sympodialis homolog; CaLip4 (XP_712408.2), Candida albicans lipase 4; AfLip (XP_753242.1), Aspergillus fumigatus lipase. Hash marks indicate the conserved lipase motif (G-X-S-X-G). Jalview 2.0 (http://www.jalview.org) was used for sequence alignment.
95.5 kDa, respectively, as predicted (Fig. 2A).

**Effects of pH on MrLip5 activity and stability**

It is well-established that pH influences skin condition; the normal skin surface is considered to be acidic, whereas the diseased skin surface becomes alkaline. We therefore investigated whether MrLip5 activity is influenced by environmental pH conditions, and enzyme activity was evaluated across varying pH values (pH 5.0 ~ 9.0) as previously described. To remove the possibility that the activity of the MrLip5 protein containing the MBP tag against the substrate, p-nitrophenyl palmitate, the activity of purified MBP alone was measured, and the values were subtracted from those of MrLip5-MBP. Our results showed

**Fig. 2.** Purification of MrLip5, and effects of pH on enzyme activity and stability. MrLip5 was fused to 6×histidine (His) and the maltose-binding protein (MBP) tag and heterologously expressed in *Escherichia coli*. The recombinant protein was purified using a His column, separated by SDS-PAGE and stained with Coomassie Brilliant Blue. MBP (42.6 kDa), MBP without MrLip5; MBP-Lip5 (95.5 kDa), MrLip5 fused with MBP (A). The relative activity and stability of MrLip5 at varying pH levels, normalized to pH 8.0. Data were obtained from four independent assays. Error bars indicate standard deviations. Purified MBP without MrLip5 was not included because it displayed no lipase activity over the pH range tested (B).

**Fig. 3.** MrLip5 expression at varying pH values. MrLip5 expression levels in *Malassezia restricta* KCTC 27527 cells grown in media of varying pH values (pH 5.0 ~ 9.0) at 34°C were assayed by western blot analysis using MrLip5-specific anti-serum (upper panel). A total of 20 μg of protein samples were loaded on SDS-PAGE gels, and membranes were stained with copper phthalocyanine-3,4,4′,4″-tetrasulfonic acid tetrasodium (CPTA) to ensure equal loading (lower panel) (A). MrLip5 expression levels in *M. restricta* strains KCTC 27524, KCTC 27529, KCTC 27539, KCTC 27540, KCTC 27543, and KCTC 27550 grown in pH 5.0 and pH 8.0 media at 34°C were assayed by western blot analysis using MrLip5-specific anti-serum (upper panel). A total of 10 μg of protein samples were loaded on SDS-PAGE gels, and membranes were stained with CPTA to ensure equivalent loading (lower panel). Representative data from two independent experiments are shown (B).
that purified MrLip5 protein possesses lipase activity, and revealed that maximum activity of the enzyme occurred at pH 7.0 (Fig. 2B). Consistent with this, the highest MrLip5 stability was observed in pH range 7.0–8.0. These results suggested that MrLip5 is most active and stable at pH 7.0–8.0, rather than acidic under conditions, below pH 7.0.

Effects of pH on expression of MrLip5

The influence of pH on MrLip5 activity led us to analyze the expression of MrLip5 at varying pH levels. MrLip5-specific antiserum was generated as described in MATERIALS AND METHODS, and was subsequently used to investigate whether expression of the lipase is regulated by pH. The result of western blot analysis showed that MrLip5 was most highly expressed in cells grown at pH 8.0 (Fig. 3A), which correlated well with the activity and stability of the enzyme. Next, we investigated its expression in other clinical isolates of M. restricta strains to rule out that the pH-dependent expression pattern of MrLip5 is specific to M. restricta KCTC 27527. All clinically isolated M. restricta strains other than M. restricta KCTC 27527 displayed higher expression levels of MrLip5 at pH 8.0 than those at pH 5.0, indicating that MrLip5 is regulated by environmental pH, and is expressed more highly under alkaline than under acidic pH (Fig. 3B).

In addition to pH, we also investigated the effect of temperature on MrLip5 expression, as this parameter is considered to be a critical environmental factor influencing skin physiology. M. restricta KCTC 27527 cells were grown at 34°C and 37°C in media with different pH levels, and expression of MrLip5 was evaluated by western blot analysis. Overall, MrLip5 expression was generally lower in cells grown at pH 5.0 than in cells grown at pH levels 7.0 or 8.0, and such an expression pattern was independent of temperature (Fig. 4). These results suggested that the effect of temperature on MrLip5 is marginal.

DISCUSSION

M. restricta possesses a total of 12 lipase genes, and among these, MrLIP5 is the most frequently expressed on dandruff scalp, suggesting the importance of MrLip5 enzyme activity at the fungal and skin surface interface. MrLip5 belongs to the LIP family (PF03583) of lipases. Interestingly, lipases that have been shown to be associated with virulence in C. albicans also belong to the LIP family lipase. In particular, MrLip5 is the most similar to CaLip4. CaLip4 is expressed in C. albicans-infected tissues during orogastic candidiasis in mice and in human patients suffering from oral candidiasis, suggesting an association of CaLip4 with virulence. Volvocine C. albicans support our hypothesis that MrLip5 plays an important role in virulence of M. restricta on the skin surfaces under different environmental conditions, specifically pH and temperature, that influence skin. Normal pH levels range from 5.0–5.9 in healthy skin, depending on anatomical site, age and gender, whereas these values can be elevated in diseased skin surfaces. Examples include elevated pH of whole-body surfaces in children with seborrheic dermatitis, atopic eczema, and xeroderma compared with normal skin pH levels. Additionally, significantly increased pH values are observed in the skin of children with atopic dermatitis compared with that of healthy children. Furthermore, pH influences activity of the lipase in various pathogenic microorganisms. For instance, the optimum pH for Rv0183, a Mycobacterium tuberculosis lipase that degrades host cell lipids to ensure its intracellular survival within phagocytes, is between 7.5 and 9.0.

The results of our study showed that both activity and stability of MrLip5 are highest under weak alkaline pH, but are significantly diminished under acidic and strong alkaline pH, suggesting that the lipase may exhibit greater activity at alkaline skin pH levels. We should note that the optimum pH of MrLip5 differed from that of another identified lipase, MrLip1, which displays highest activation and expression at pH 5.0, indicating that MrLip5 and MrLip1 act under different skin conditions and may play different roles. In addition, we showed that the expression of MrLip5 was higher under alkaline pH conditions than under acidic pH. These results suggest that the expression and enzyme activity of MrLip5 are pH-dependent and that pH-dependent regulatory mechanisms may govern the expression of lipases in M. restricta. In C. albicans, signal transduction pathways and the tran-
scription factor Rim101 governs sensing and responding to ambient pH. Therefore, we assume that similar transcriptional regulatory mechanisms may exist in *M. restricta*.

Moreover, the effect of temperature on the expression of MrLip5 was also different from that on MrLip1 expression. In *M. restricta* cells grown at pH 5.0, MrLip5 exhibited lower expression levels than those in cells grown at pH 7.0 or 8.0. Considering that pH 5.0 and 34°C likely represent the normal human skin condition, MrLip5 might be expressed to a lesser extent in the skin surfaces of healthy individuals. Taken together with our previous results regarding the expression of *MrLIP5* transcripts in subjects with severe dandruff, our findings suggest a potential role of MrLip5 in skin disease progression involving *M. restricta*.

We are aware that further functional characterization of MrLip5, including virulence assays, remains to be carried out. Nevertheless, we believe that our current data provide basic information to improve the understanding of the roles of *M. restricta* lipases in the progression of skin diseases.

**CONFLICTS OF INTEREST**

The authors have nothing to disclose.

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**DATA SHARING STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**REFERENCES**

1. Wang L, Clavaud C, Bar-Hen A, Cui M, Gao J, Liu Y, et al. Characterization of the major bacterial-fungal populations colonizing dandruff scalps in Shanghai, China, shows microbial disequilibrium. Exp Dermatol 2015;24:398-400.
2. Clavaud C, Jourdain R, Bar-Hen A, Tichit M, Boucher C, Pouradier F, et al. Dandruff is associated with disequilibrium in the proportion of the major bacterial and fungal populations colonizing the scalp. PLoS One 2013;8:e58203.
3. Crespo-Erchiga V, Gómez-Moyano E, Crespo M. [Ptyriasis versicolor and the yeasts of genus Malassezia]. Actas Dermosifiliogr 2008;99:764-771. Spanish.
4. Patiño-Uzcátegui A, Amado Y, Cepero de García M, Chaves D, Tabima J, Motta A, et al. Virulence gene expression in Malassezia spp from individuals with seborrheic dermatitis. J Invest Dermatol 2011;131:2134-2136.
5. Casagrande BF, Flückiger S, Linder MT, Johansson C, Schemynius A, Cramer R, et al. Sensitization to the yeast Malassezia sympodialis is specific for extrinsic and intrinsic atopic eczema. J Invest Dermatol 2006;126:2414-2421.
6. Xu Z, Wang Z, Yuan C, Liu X, Yang F, Wang T, et al. Dandruff is associated with the conjoined interactions between host and microorganisms. Sci Rep 2016;6:24877.
7. Cabañes FJ, Coutinho SD, Puig L, Bragulat MR, Castellá G. New lipid-dependent Malassezia species from parrots. Rev Iberoam Micol 2016;33:92-99.
8. Honnavar P, Prasad GS, Ghosh A, Dogra S, Handa S, Rudramurthy SM. Malassezia arunalokei sp. nov., a novel yeast species isolated from seborrheic dermatitis patients and healthy individuals from India. J Clin Microbiol 2016;54:1826-1834.
9. Park T, Kim HJ, Myeong NR, Lee HG, Kwack I, Lee J, et al. Collapse of human scalp microbiome network in dandruff and seborrheic dermatitis. Exp Dermatol 2017;26:835-838.
10. Kato H, Sugita T, Ishibashi Y, Nishikawa A. Detection and quantification of specific IgE antibodies against eight Malassezia species in sera of patients with atopic dermatitis by using an enzyme-linked immunosorbent assay. Microbiol Immunol 2006;50:851-856.
11. Xu J, Saunders CW, Hu P, Grant RA, Boekhout T, Kuramae EE, et al. Dandruff-associated Malassezia genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens. Proc Natl Acad Sci U S A 2007;104:18730-18735.
12. Wu G, Zhao H, Li C, Rajapakse MP, Wong WC, Xu J, et al. Genus-wide comparative genomics of Malassezia delineates its phylogeny, physiology, and niche adaptation on human skin. PLoS Genet 2015;11:e1005614.
13. Gioti A, Nystedt B, Li W, Xu J, Andersson A, Averette AF, et al. Genomic insights into the atopic eczema-associated skin commensal yeast Malassezia sympodialis. mBio 2013;4:e00572-12.
14. Park M, Cho YJ, Lee YW, Jung WH. Whole genome sequencing analysis of the cutaneous pathogenic yeast Malassezia restricta and identification of the major lipase expressed on the scalp of patients with dandruff. Mycoses 2017;60:188-197.
15. Cho YJ, Park M, Jung WH. Resequencing the genome of Malassezia restricta Strain KCTC 27527. Microbiol Resour
16. Wong H, Schotz MC. The lipase gene family. J Lipid Res 2002;43:999-999.
17. Sommer B, Overy DP, Kerr RG. Identification and characterization of lipases from Malassezia restricta, a causative agent of dandruff. FEMS Yeast Res 2015;15:e0078.
18. Sommer B, Overy DP, Haliti B, Kerr RG. Secreted lipases from Malassezia globosa: recombiant expression and determination of their substrate specificities. Microbiology (Reading) 2016;162:1069-1079.
19. Ro BI, Dawson TL. The role of sebaceous gland activity and scalp microfloral metabolism in the etiology of seborrheic dermatitis and dandruff. J Invest Dermatol Symp Proc 2005;10:194-197.
20. DeAngelis YM, Gemmer CM, Kaczvinsky JR Jr, Kenneally DC, Schwartz JR, Dawson TL Jr. Three etiologic facets of dandruff and seborrheic dermatitis: Malassezia fungi, sebaceous lipids, and individual sensitivity. J Invest Dermatol Symp Proc 2005;10:295-297.
21. Schofield DA, Westwater C, Warner T, Balish E. Differential Candida albicans lipase gene expression during alimentary tract colonization and infection. FEMS Microbiol Lett 2005;244:359-365.
22. Stehr F, Felk A, Gácsé R, Kretschmar M, Mühns B, Neuber K, et al. Expression analysis of the Candida albicans lipase family gene during experimental infections and in patient samples. FEMS Yeast Res 2004;4:401-408.
23. Fu Y, Ibrahim AS, Fonzi W, Zhou X, Ramos CF, Ghannoum MA. Cloning and characterization of a gene (LIP1) which encodes a lipase from the pathogenic yeast Candida albicans. Microbiology (Reading) 1997;143(Pt 2):331-340.
24. Hube B, Stehr F, Bossenz M, Mazur A, Kretschmar M, Schäfer W. Secreted lipases of Candida albicans: cloning, characterisation and expression analysis of a new gene family with at least ten members. Arch Microbiol 2000;174:362-374.
25. Voigt CA, Schäfer W, Salomon S. A secreted lipase of Fusarium graminearum is a virulence factor required for infection of cereals. Plant J 2005;42:364-375.
26. Brunke S, Hube B. MLIP1, a gene encoding an extracellular lipase of the lipid-dependent fungus Malassezia furfur. Microbiology (Reading) 2006;152(Pt 2):547-554.
27. Shibata N, Okamura N, Hirai K, Arikawa K, Kimura M, Okawa Y. Isolation, characterization and molecular cloning of a lipolytic enzyme secreted from Malassezia pachydermatis. FEMS Microbiol Lett 2006;256:137-144.
28. DeAngelis YM, Saunders CW, Johnstone KR, Reedor NL, Coleman CG, Kaczvinsky JR Jr, et al. Isolation and expression of a Malassezia globosa lipase gene, LIP1. J Invest Dermatol 2007;127:2138-2146.
29. Juntachai W, Oura T, Kajiwara S. Purification and characterization of a secretory lipolytic enzyme, MgLIP2, from Malassezia globosa. Microbiology (Reading) 2011;157(Pt 12):3492-3499.
30. Park M, Jung WH, Han SH, Lee YH, Lee YW. Characterisation and expression analysis of MrLip1, a Class III family lipase of Malassezia restricta. Mycoses 2015;58:671-678.
31. Ali S, Khan FI, Mohammad T, Lan D, Hassan ML, Wang Y. Identification and evaluation of inhibitors of lipase from Malassezia restricta using virtual high-throughput screening and molecular dynamics studies. Int J Mol Sci 2019;20:884.
32. Leenming JP, Notman FH. Improved methods for isolation and enumeration of Malassezia furfur from human skin. J Clin Microbiol 1987;25:2017-2019.
33. Berrow NS, Alderton D, Sainsbury S, Netleship J, Assenberg R, Rahman N, et al. A versatile ligation-independent cloning method suitable for high-throughput expression screening applications. Nucleic Acids Res 2007;35:e45.
34. Juntachai W, Oura T, Murayama SY, Kajiwara S. The lipolytic enzymes activities of Malassezia species. Med Mycol 2009;47:477-484.
35. Lan DM, Yang N, Wang WK, Shen YF, Yang B, Wang YH. A novel cold-active lipase from Candida albicans: cloning, expression and characterization of the recombinant enzyme. Int J Mol Sci 2011;12:3950-3965.
36. Cheng YY, Qian YK, Li ZF, Wu ZH, Liu H, Li YZ. A novel cold-adapted lipase from Sorangium cellulosum strain So0157-2: gene cloning, expression, and enzymatic characterization. Int J Mol Sci 2011;12:6765-6780.
37. Djordjevic JT, Del Poeta M, Sorrell TC, Turner KM, Wright LC. Secretion of cryptococcal phospholipase B1 (PLB1) is regulated by a glycosylphosphatidylinositol (GPI) anchor. Biochim Biophys Acta 2005;1705(3):91-105.
38. Siafakas AR, Sorrell TC, Wright LC, Wilson C, Larsen M, Boadle R, et al. Cell wall-linked cryptococcal phospholipase B1 is a source of secreted enzyme and a determinant of cell wall integrity. J Biol Chem 2007;282:37508-37514.
39. Bartosch E, Bartosch EM. Secretion of a newly identified lipase from Malassezia restricta using virtual high-throughput screening and analysis workbench. Bioinformatics 2009;25:1189-1191.
40. Nierman WC, Pain A, Anderson MJ, Wortman JR, Kim HS, Arroyo J, et al. Genomic sequence of the pathogenic and allergenic filamentous fungus Aspergillus fumigatus. Nature 2005;438:1151-1156.
41. Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 2009;25:1189-1191.
42. Jung YC, Kim EJ, Cho JC, Suh KD, Nam GW. Effect of skin pH for wrinkle formation on Asian: Korean, Vietnamese and Singaporean. J Eur Acad Dermatol Venereol 2013;27:e328-e332.
43. Anderson DS. The acid-base balance of the skin. Br J Dermatol 1951;63:283-296.
44. Eberlein-König B, Schäfer T, Huss-Marp J, Darsow U, Boadle R, et al. Cell wall-linked cryptococcal phospholipase B1 is a source of secreted enzyme and a determinant of cell wall integrity. J Biol Chem 2007;282:37508-37514.
46. Sparavigna A, Setaro M, Gualandri V. Cutaneous pH in children affected by atopic dermatitis and in healthy children: a multicenter study. Skin Res Technol 1999;5:221-227.
47. Seidenari S, Giusti G. Objective assessment of the skin of children affected by atopic dermatitis: a study of pH, capacitance and TEWL in eczematous and clinically uninvolved skin. Acta Derm Venereol 1995;75:429-433.
48. Côtes K, Dhouib R, Douchet I, Chahinian H, de Caro A, Carrière F, et al. Characterization of an exported monoglyceride lipase from Mycobacterium tuberculosis possibly involved in the metabolism of host cell membrane lipids. Biochem J 2007;408:417-427.
49. Davis D. Adaptation to environmental pH in Candida albicans and its relation to pathogenesis. Curr Genet 2003; 44:1-7.