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Evaluation of Antifungal Activities of Bee Venom Components Against *Malassezia* Strains

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Background: Several studies about bee venom components have reported anti-fungal, anti-inflammatory, and anti-cancer effects. *Malassezia* species are components of skin flora, and also associated with many dermatologic diseases such as seborrheic dermatitis, pityriasis versicolor, folliculitis, and atopic dermatitis.

Objective: To investigate the antifungal activity of bee venom components, melittin, and apamin, against *Malassezia* strains.

Methods: With 10 *Malassezia* species, minimal inhibitory concentrations (MICs) were tested with bee venom, and Zinc pyrithione as a reference antifungal agent.

Results: Whole bee venom, melittin, and apamin did not show any antifungal activity against *Malassezia* species at the concentrations tested.

Conclusion: Although bee venom do not have anti-*Malassezia* activities, it is known to have antifungal activities against other fungal strains. Therefore, further study should consider revealing the mechanism of antifungal activity against fungus and other possible target strains of fungi.

Key Words: Antifungal activity, Bee venom, *Malassezia*, Scalp, Seborrheic dermatitis

INTRODUCTION

Malassezia species, a major element of the skin microbiome, are lipophilic yeasts that usually inhabit the face and scalp[1,2]. These species are strongly associated with many dermatologic and systemic diseases, including pityriasis versicolor, seborrheic dermatitis (SD), folliculitis, atopic dermatitis, and systemic infections[1-3].

Antifungal agents against *Malassezia* are important components of seborrheic dermatitis treatment[4]. There are many anti-*Malassezia* drugs, but due to inadequate or irregular use of the drugs, resistance to antifungal agents has been increasing. Therefore, there is a need to identify and develop novel agents for use against *Malassezia* species.

For many decades, bee venom (BV) therapy has been used as a traditional medicine to treat various diseases, such as

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cancer, arthritis, chronic pain, rheumatism, and skin diseases[5-7]. BV is composed of many different types of peptides, such as melittin, apamin, adolapin, and also unknown chemicals. Several studies on effects of BV have reported that they possess are anti-tumor, anti-inflammatory, anti-arthritis, and also antifungal properties[5-8].

Melittin, one of the major components of BV, is a small protein consisting of 26 amino acids, and its various biological effects have been studied in anti-inflammatory, anti-tumor, and anti-arthritis therapy[9,10]. However, melittin also shows toxic properties such as hemolysis, anaphylaxis, and other unknown side effects, warranting further investigation to optimize its therapeutic effect with minimum toxicity.

Apamin is a protein of 10 amino acids, and it is a selective inhibitor of the calcium dependent potassium channels. Apamin's structural and pharmacological effects may play a cytotoxic role in cancer cells and other inflammatory cells[10-12].

Previous studies have reported the antifungal activities of BV against *Candida* species and *Trichophyton rubrum*[10,13]. In the current study, we investigated if BV shows anti-*Malassezia* effects and examined the potential role of its two main components, melittin and apamin.

MATERIALS AND METHODS

This study was conducted with 10 *Malassezia* yeast strains. Ten *Malassezia* strains are consist of *M. restricta*, which is observed predominantly from the scalp and forehead, and *M. globosa*, which is observed predominantly from the front and chest, and *M. sympodialis*, and these 3 species are typically referred as the causative fungi for scalp seborrheic dermatitis. MIC results for each strain were obtained through three independent experiments.

1. BV collection and processing of the components

We used one species of bee, *Apis mellifera L.*, which were kept at the National Academy of Agricultural Science, Korea. Collecting device of BV (Chung Jin Biotech Co., Ltd., Korea) provided an electric current flow through to the hive causing the bees to sting at the plate. The collected venom was lyophilized by freeze dryer and kept at 4℃ for preservation. The BV used in this study was confirmed with size exclusion gel chromatography (AKTA Explorer; GE Healthcare, Pittsburgh, PA, USA) by dissolving in 0.02 M phosphate buffer. We used manufactured melittin and apamin products from BBM (A.C. Care Bee' water essence) of Dongsung Pharmaceuticals (Seoul, Korea) and Sigma (St. Louis, MO, USA).

2. Minimal inhibitory concentration (MIC) measurement

The antifungal activity was evaluated against 3 strains of *Malassezia restricta*, 3 strains of *M. globosa*, *M. pachydermatis*, *M. slooffiae*, *M. sympodialis*, and *M. furfur*. The strains were grown in Leeming and Notman agar (15 g agar, 10 g peptone,

Table 1. Minimal inhibitory concentrations of *Malassezia* species

Malassezia strains	Tested compounds (μg/mL)			
	Zinc pyrithione	Bee venom	Melittin	Apamin
M. restricta CBS 7877	1.59	>100	>20	>20
M. restricta KCTC 27524	1.59	>100	>20	>20
M. restricta KCTC 27527	1.59	>100	>20	>20
M. globosa CBS 7899	1.59	>100	>20	>20
M. globosa KCTC 27511	1.59	>100	>20	>20
M. globosa KCTC 27520	1.59~3.18	>100	>20	>20
M. sympodialis CBS 7222	1.59	>100	>20	>20
M. slooffiae KCTC 27517	3.18	>100	>20	>20
M. furfur CBS 1878	3.18	>100	>20	>20
M. pachydermatis CBS 1879	3.18	>100	>20	>20

10 g glucose, 8.0 g desiccated bile, 2.0 g yeast extract, 0.5 g glycerol monostearate, 10 mL glycerol, 5.0 mL Tween 60, and 20 mL olive oil). MICs were determined with concentrations of BV, melittin, apamin, and zinc pyrithione (ZPT; reference antifungal agent) ranging from 6.25 to 100 μg/mL, 1.25 to 20 μg/mL, 1.25 to 20 μg/mL, and 0.4 to 636 μg/mL, respectively. Inoculated 50 μL cell suspension on each surface of agar plate is incubated for 3 days. MIC test was repeated three times.

RESULTS

MICs of BV, melittin, apamin, and ZPT were measured for each strain. ZPT is one of the most commonly used anti-*Malassezia* treatment agent and was used as a control. The results of independent experiments are listed in Table 1. The MIC value of ZPT was lowest at 1.59 μg/mL for *M. retricta*, *M. globosa*, and *M. sympodialis*. MIC values ranged from 1.59 to 3.18 μg/mL for strains of *M. globosa* KCTC 27520. For *M. slooffiae*, *M. furfur* and *M. pachydermatis*, relatively high MIC of 3.18 μg/mL was observed. For BV, all strains showed MIC values of > 100 μg/mL and for both melittin and apamin, all strains showed MIC values of > 20 μg/mL.

DISCUSSION

SD is an inflammatory disease of the scalp, showing chronic course, and affecting approximately 5~10% of total population[1,4]. Common symptoms of SD are itching sensation, scales, and redness. Severe and chronic form of SD can result in permanent hair loss, which negatively affects the quality of life of patients[14]. The pathogenesis of SD is not fully understood, but there is a strong connection between *Malassezia* yeast and SD. One of the hypotheses is an interaction between *Malassezia*, keratinocytes, and immune system that results transforms the *Malassezia* species into a pathogen[15]. These pathogenic reactions lead to disruption of the epidermal barrier and trigger inflammatory reactions. Furthermore, these changes make keratinocytes produce pro-inflammatory cytokines, continuing the inflammatory status of SD. Thus, decreasing inflammation and inhibiting *Malassezia* activities would be the goal of SD treatment.

In previous study, disease severity of SD was correlated with the population and number of microorganisms, including *Malassezia* species[16,17]. Thus, decreasing the number of *Malassezia* yeasts in the lesion is important for SD treatment. According to recent studies, antifungal drug resistance has become an issue causing treatment failure and relapses of fungal diseases. In this study, we tried to identify a new antifungal agent effective against *Malassezia* species, by investigating BV components, which is actively being researched currently.

In previous studies, BV was shown to have antifungal effects against *Trichophyton rubrum* and *Candida albicans*. A study showed raw BV inhibited growth of *T. rubrum*, but melittin or apamin did not show any significant inhibition[13]. Another study revealed that BV has antifungal effects against 10 subtypes of *C. albicans*, which were isolated from human vaginal wall and blood[10]; the corresponding MIC values were 62.5 μg/mL to 125 μg/mL for BV. However, our study showed that BV is not effective as an antifungal agent. Nonetheless, we have to consider the structural and metabolic differences between *T. rubrum* and *Malassezia* species. *Malassezia* encode many secretory genes of aspartyl proteases, lipases, acid spingomyelinases, and phospholipase C than that of *Trichophyton* and *Candida* species, and these enzymes can disintegrate the substances that act as antifungals[4]. Further studies are necessary, including more specific associated enzyme analysis and mechanism of antifungal activity of BV on *T. rubrum* and *C. albicans*.

There are some limitations of our study. First, result of MIC was not always correlated with clinical outcome of antifungal treatment. This study was performed only as an *in vitro* demonstration of resistance, which cannot be equated to *in vivo* resistance. The determinants of the resistance are mostly host-related factors, including the affected site, formula of the antifungal agent, severity of diseases, and other mycobiome on the skin. BV and its components, melittin and apamin, have anti-inflammatory effects, and so may have beneficial effects on chronic inflammatory status of SD patient's scalp [5,18-20]. Second, diluted concentrations used in this study may have been insufficient to show an effect, which may require higher concentrations. However, we should consider the adverse effects that bee venom may have[21]. Considering this, further studies should aim on targeting optimized concentration of the agent. Third, we lacked sufficient information to evaluate the quality control of this experiment; there are no reported references of MIC values for BV against *Malassezia* yeasts.

Further studies should confirm the effects of BV and its derivatives on fungi species and the associated mechanism. It is also necessary to identify the active antifungal components in BV, which can aid in formulating effective agents for fungal infections such as *T. rubrum* and *Candida* infection. On the other hand, it is necessary to figure out the functional and structural features protecting *Malassezia* species from BV.

This study provides the basis for further research for the development of new antifungal agent using BV and its components.

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CONFLICT OF INTEREST

In relation to this article, we declare that there is no conflict of interest.

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