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Oral presentation

Microbiological Evaluation of Perga (bee bread)

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Abstract:

Bee bread has rich nutrient content and probiotic properties and, is widely used in traditional medicine. We aimed to investigate whether there is difference between fresh and stored perga in terms of microbiological reproduction, and susceptibility of isolated strains. The 34 strains were isolated in taken fresh perga samples. No microorganism was growth in stored perga samples. The isolated strains were identified by conventional and MALDI-TOF MS methods. The 34 strains were identified as *Aspergillus spp.* (n:12), *Rhizopus oryzae* (n:6), *Mucor circinelloides* (n:1), *Bipolaris* (n:2), *Trichoderma* (n:3), *Paecilomyces variotii* (n:1), *Penicillium chrysogenum* (n:1), *Kodamaea ohmeri* (n:1), *Bacillus altitudinis/pumilus* (n:3), *Bacillus licheniformis* (n:1), *B. megaterium* (n:1), *Micrococcus luteus* (n:1) and *Serratia marcescens* (n:1). MICs values of itraconazole, voriconazole, anidulafungin and caspofungin for *Mucor* and *Rhizopus* strains had high (≥ 32 $\mu\text{g/ml}$) except amphotericinB, posaconazole. MICs values of drugs for *Aspergillus* strains (n:9/12) were low (1 $\mu\text{g/ml}$). *Trichoderma* strains had low MIC values (≤ 0.50 $\mu\text{g/ml}$) for other drugs except itraconazole. *P. chrysogenum* was found to have low MIC value (≤ 0.25 $\mu\text{g/ml}$) for POS, AND, CS. *Bipolaris*, *P. variotii* and *K. ohmeri* was had low MIC values to all antifungals.

Key words: Bee bread, bacteria, fresh or stored, molds, susceptibility, yeast.

Introduction

Consumers could assume that pollen and bee bread are similar bee products; however, bee bread is more valuable compare to pollen because of the result of lactic acid fermentation of combined product by pollen, nectar, and bee salivary enzymes by microorganisms such as bacteria (*Pseudomonas spp.* and *Lactobacillus spp.*) or yeasts (*Saccharomyces spp.*) or both and is characterized by a wide range of biological properties (Vasquez and Olofsoni 2009; Bakour 2019). Also, bee bread can be affected by the microbiota of bees as it contains the secretions of bees salivary glands. Bee bread differs from pollen in terms of enzymes, lactic acid, sugar, starch, protein, and carbohydrate content. Bee bread is including peptides, free amino acids, phenolic compounds, minerals, B vitamins and vitamin K and, is more easily absorbed in the human body. All microorganisms in bee bread have been studied; however, any study about whether there is a difference in microbiological reproduction between long-stored and fresh bee bread was not come across. In this study, we aimed to investigate that there is a difference between fresh and long stored (one year) bee bread (perga) in terms of microbiological reproduction and susceptibility of isolated strains or not.

Material and Methods

Gradient diffusion test (E-test strips) and broth microdilution method were used for antifungal susceptibility testing. The MIC values of itraconazole, voriconazole, amphotericin B,



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fluconazole and ketoconazole were determined by the broth microdilution method. The MIC values of posaconazole (POS), anidulafungin (AND) and caspofungin (CS) were determined by the gradient diffusion test (E-test strips). The fluconazole and ketoconazole susceptibility were investigated only in yeasts strains. The *in vitro* antifungal activity of antifungal drugs was investigated by the following guidelines Clinical & Laboratory Standards Institute (CLSI) M27-S4 for yeast and CLSI M38-A2 for molds. The *in vitro* antibiotic susceptibility patterns of the bacterial isolates were determined by disc diffusion method, and the results were interpreted according to CLSI

RPMI 1640 medium with L-glutamine, without sodium bicarbonate and phenol red (Sigma-Aldrich, UK), buffered with 34.53 g MOPS (3-N- morpholinepropanesulfonic acid) (PanReac & AppliChem, USA), pH7.0, was used for the broth microdilution test. Two-fold dilutions of the drugs were performed and distributed in 96-well flat-bottom plates in concentrations ranging from 64–0.125 µg/mL for FLU, 16–0.03 µg/mL for AP, IT, VO, and KTZ. The fungal inoculum was prepared from a 24 h SDA (Oxoid, UK) culture incubated at 35°C, and mold inoculum suspensions were prepared from well-sporulated cultures grown on potato dextrose agar and adjusted spectrophotometrically to a turbidity. The plates were incubated at 35°C for 24–48 h. The minimal inhibitory concentrations (MIC) of the drugs were determined according to the CLSI recommendations.

Results

Yeast, molds and bacteria were isolated from fresh bee bread samples. The 34 strains were isolated from 8 different hives, 26 of them were mold, one was yeast and seven were bacteria. Anaerobic microorganism was not isolated from fresh bee bread samples in our study. The 34 strains were identified as *Aspergillus niger* complex (n: 5), *Aspergillus fumigatus* (n:1), *Aspergillus nidulans* (n:2), *Aspergillus terreus* (n:2), *Aspergillus flavus* (n:2), *Rhizopus oryzae* complex (n:6), *Mucor circinelloides* (n:1), *Bipolaris spp.* (n:2), *Trichoderma spp* (n: 3), *Paecilomyces variotii* (n: 1), *Penicillium chrysogenum* (n:1), *Kodamaea ohmeri* (n:1), *Bacillus altitudinis/pumilus* (n:3), *Bacillus licheniformis* (n: 1), *Bacillus megaterium* (n: 1), *Micrococcus luteus* (n: 1) and *Serratia marcescens* (n:1) according to the conventional culture methods and MALDI-TOF MS.

Discussion

We isolated yeast, mold and bacteria from fresh bee bread samples and observed that these isolated strains could be resistant to antibiotics and antifungal drugs. When we look at the literatures, we know that these strains may cause serious infections and high mortality in immunocompromised individuals. According to our results, it was seen that patients with immune deficiency would not be suitable to consume fresh bee bread. Another remarkable point in our study was the lack of yeast, mold and other microorganisms in the stored bee bread sample.

References

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