Molecular typing of *Malassezia restricta*, *Malassezia arunalokei* and *Malassezia globosa* isolated from seborrhoeic dermatitis/dandruff patients and healthy controls by FAFLP

Prasanna Honnavar,^{#a} Arunaloake Chakrabarti,^a Sunil Dogra,^b PVM Lakshmi,^c Shivaprakash M. Rudramurthy^a

[#] Department of Microbiology and Immunology, Xavier University School of Medicine, Oranjestad, Aruba

^aDepartment of Medical Microbiology, ^bDepartment of Dermatology, Venerology, & Leprosy and ^cDepartment of Community Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Introduction

- The commensal *Malassezia* yeasts are associated with several dermatological disorders including seborrhoeic dermatitis/dandruff (SD/D).
- M. globosa* and *M. restricta* are the predominant agents associated with SD/D. *M. arunalokei*, newly described species was isolated only from Indian SD patients.
- Malassezia* research in association with SD/D was held back, due to the unavailability of these difficult to culture species.
- The purpose of study was to differentiate *Malassezia* strains causing SD/D lesions from those which exist as commensal using a highly discriminatory DNA fingerprinting technique, Fluorescent Amplified Fragment Length Polymorphism (FAFLP).

Method

- A total of 200 patients having different degree of SD/D and 100 healthy individuals from Urban and Rural areas of north India were included in the study. Dandruff scales and skin swab were collected from the scalp and nasolabial folds respectively.
- Malassezia* spp. isolated were identified by conventional method and confirmed by ITS2-PCR-RFLP and sequencing of ITS-region of rDNA. Molecular typing of a large number of clinical isolates [n=154 (*M. globosa*, n=85; *M. restricta*, n=55; *M. arunalokei*, n=14) and 3 standard strains] from our study group was analyzed using FAFLP.
- DNA sample was digested with restriction enzymes EcoRI and HindIII, and the fragments were linked to prepared adapters.
- Pre-selective followed by selective amplification reactions were performed using EcoRI-AC (6-carboxyfluorescein [6-FAM] labeled) and HindIII-T selective primers. The amplified fragments were separated by capillary electrophoresis on an ABI3130 sequencer.
- The similarity coefficient was determined by Pearson correlation with negative similarities clip to zero. The densitometric curve cluster analysis was performed by unweighted pair group method with arithmetic means using Bionumerics software. The similarity coefficient of 70% was taken as cut-off.
- Due to high number of strains, *M. restricta* and *M. arunalokei* strains were analyzed together. *M. globosa* was analyzed separately. *M. furfur* was used as an out-group.

Results

- M. restricta* and *M. arunalokei* isolates had similarity co-efficient of 20%, whereas it was 50% among *M. restricta* and 60% among *M. arunalokei* isolates.
- Majority of the isolates (n=38,69%) clustered to form FAFLP type-I, followed by type-II (n=14,25%) and type-III (n=3,6%). All three FAFLP types formed tight clustering among themselves (>80% similarity co-efficient) showing less genetic diversity within each FAFLP type. Type-II clustered strains were isolated from the scalp of SD/D patients (p<0.05).
- M. arunalokei* isolates were clustered into two FAFLP types (p=0.05). All FAFLP type-I isolates (n=10,71%) were from the scalp of moderate and severe SD/D patients, whereas all type-II isolates (n=4,29%) were from nasolabial folds of either SD/D patients or healthy controls.
- All 11 FAFLP types of *M. globosa* formed very loose structured cluster showing high genetic diversity in intra and inter-FAFLP types. Majority of the isolates (n=63,74%) clustered to form FAFLP type-II, followed by type X (n=5,6%), type-I (n=4,5%), type-III (n=3,3.5%), type-IV (n=3,3.5%), type-V (n=2,2%), type-VIII (n=2,2%) and type-VI, VII, IX, XI (n=1,1% each).
- No geographic specific isolate clustering was observed in FAFLP

Conclusion

M. restricta FAFLP type-II and *M. arunalokei* FAFLP type-I strains were exclusively isolated from the scalp of moderate to severe form of SD/D. However, no such severity specific clustering was observed in *M. globosa*. The results strongly suggest the role of particular genotypes of *M. restricta* and *M. arunalokei* in causation of SD/D.

FAFLP banding pattern of *M. restricta* and *M. arunalokei* strains

FAFLP banding pattern of *M. globosa* strains

References

- Gupta AK et al, JCM- 2004, 4253-60
- Cabanes et al, FEMS- 2007, 1064-1076
- Honnavar P. et al, JCM- 2016, 1826-1834
- Chakrabarti A et al, CMI-2014, 83-90