ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 4, pp. 16624-16631, April, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

CHARACTERIZATION OF *MALASSEZIA* SPECIES ISOLATED FROM PITYRIASIS VERSICOLOR PATIENTS AND HEALTHY SUBJECTS OF NORTH-EAST INDIA BY PCR-RFLP AND 26SrDNA SEQUENCING

***Sharma, A[1]., Rabha, D[1]., Hazarika, D[2]., Saikia, A[3] and Ahmed, G[4]**

[1]Department of Microbiology, Gauhati Medical College, Guwahati, Assam, India
[2]Department of Dermatology, Venereology and Leprology, Gauhati Medical College, Guwahati, Assam, India
[3]Department of Community Medicine, Gauhati Medical College, Guwahati, Assam, India
[4]Department of Biotechnology, Gauhati University, Guwahati, Assam, India

DOI: http://dx.doi.org/10.24327/ijrsr.2017.0804.0186

ARTICLE INFO

Article History:

Received 18th January, 2017
Received in revised form 10th February, 2017
Accepted 06th March, 2017
Published online 28th April, 2017

Key Words:

Malassezia, pityriasis versicolor, North-east India, PCR-RFLP, Healthy subjects, Sequencing

ABSTRACT

The genus *Malassezia* has gained importance as an opportunistic yeast for its association with many diseases ranging from pityriasis versicolor (PV) to invasive mycosis. Previous studies reported interesting geographical variations in prevalence of *Malassezia* species. The distribution of this emerging yeast in north-east India has not been fully explored. Hence, the present study aimed to determine distribution of *Malassezia* species in PV patients and healthy individuals in north-east India. The study included 200 PV patients and 200 healthy individuals. After isolation, *Malassezia* species were identified by phenotypic and molecular methods. The mean colony count of *Malassezia* species were significantly higher in lesional than non-lesional areas of PV patients. The predominant species was *M. furfur* (72.8%) followed by *M. globosa* (14.9%). *M. furfur* (92.5%) predominated in healthy skin also. Distribution of *Malassezia* species in PV lesions and healthy skin in north-east India varies significantly from many geographical regions across the world.

INTRODUCTION

The genus *Malassezia* is classified under the order *Malasseziales* which includes basidiomycetous yeasts without ballistospores (Moore 1980; Batra *et al.* 2005; Crespo-Erchiga and Guého 2005). They are obligatory or non-obligatory lipid dependant yeasts and are known to be a component of the commensal flora of skin of warm blooded animals (Abou-Gabal *et al.* 1979; Xhengappa *et al.* 1983; Guého *et al.* 1998 and Raabe *et al.* 1998). Recently, this genus is gaining importance as emerging pathogen as they are found to be associated with blood stream infection in immunocompromised patients and neonates receiving parenteral lipid emulsion (Crespo *et al.* 2000 and Gupta *et al.* 2014).

The fungal etiology of pityriasis versicolor was first recognized in 1846 (Eichstedt 1846). The unstable morphological variations, strict lipid dependency lead to difficulty in growing this fungus, thereby limiting study on this fungus (Guillot *et al.* 1996; Midgley 2000; Gupta *et al.* 2001 and Ashbee and Evans 2002). Reclassification of this genus included seven species namely *M. furfur*, *M. pachydermatis, M. sympodialis, M. globosa, M. obtusa, M. restricta* and *M. slooffiae* (Gupta *et al.* 2000). Currently, another eight species comprising *M. dermatis, M. japonica, M. nana, M. yamatoensis, M. caprae, M. equina, M. cuniculi* and *M. arunalokei* have been included in this genus (Guého *et al.* 1996; Sugita *et al.* 2004; Cabanes *et al.* 2011 and Honnavar *et al.* 2016).

Although PV is the only human disease for which *Malassezia* has been fully established as pathogen, debate still remains over which species or indeed whether more than one species is involved in the disease (Zhang *et al.* 2013). After recognition of the genus *Malassezia* as potential pathogen associated with a number of human diseases, there is a growing need for extensive research on this fungus. The reported literatures have shown interesting geographical variations in species distribution (Nakabayashi *et al.* 2000 and Cafarichia *et al.* 2011). The north-east region of India experiences a humid subtropical climate which creates fertile ground for many

Corresponding author: **Sharma, A*
Department of Microbiology, Gauhati Medical College, Guwahati, Assam, India

fungal pathogens. Also, the region is inhabited by population with wide socio-cultural diversity including various ethnic groups and migrant population of different states of India. Hence, it is hypothesized that, the geographical, climatic and ethnic variation can contribute towards the differences in the distribution of the *Malassezia* species in north-east India. The present study aimed to determine the distribution of *Malassezia* species in PV and healthy individuals of north-east India and their correlation with various demographic and clinical characteristics**.**

MATERIALS AND METHODS

Location and duration of the study

The study was carried out in the Department of Microbiology, Gauhati Medical College, Assam, India during a period of four years.

Study design

The study was a hospital based unmatched case control study. The study has been approved by the institutional ethics committee and was performed in accordance with the ethical standards as laid down in the Indian Council of Medical Research's Ethical guidelines for biomedical research on human participants (2006).

Sample size

Sample size was determined with continuous correction by assuming two sided confidence level (1-α) 95%, power (1-β) 80%, taking ratio of case with control 1:1, with proportion of control exposed 7.4% and assuming to detect least extreme odds ratio of 2.25. For calculating the sample size, the prevalence rate of PV in West Bengal 15.2 % was considered, as no published data is available from Assam) (Das *et al.*, 2009).

Study population

Two groups of study participants i.e. cases and control based on presence or absence of dermatosis were identified in the Department of Dermatology, Venereology and Leprology of Gauhati Medical College, Assam, India by clinical examination. Cases were selected from patients attending hospital in sequence after applying inclusion and exclusion criteria and in the similar manner controls were also recruited after taking written consent. Inclusion criteria for cases were scaling hypo- or hyperpigmented macules on various parts of the body who were not on antifungal therapy for last one month (Roberts 1969 and Crespo-Erchiga and Delgado Florencio 2002).

Selection criteria for cases and controls
Inclusion criteria for the cases (Hay *et al.*, 2004):

Patients with clinical presentation compatible with PV or clinically diagnosed as PV

- Dyschromic macules (hypo or hyperpigmented) with or without scaling.
- Patchy lesions with varying changes of skin colour.
- Patients of all ages and of both sexes.

Inclusion criteria for controls

- Patients without any clinically evident skin diseases.

Exclusion criteria for cases

- Patient with other pigmentation disorders.
- Patient with a history of antifungal therapy (systemic and topical) taken within previous one month.
- Patient with a history of topical or systemic steroid treatment within previous one month.

Exclusion criteria for controls

- Presence of dermatosis or skin diseases.

Sample collection

Scotch tape of about one square inch was applied on the skin of lesional and non-lesional sites of 200 PV cases after identification of the lesions by Wood's lamp examination, left in place for a minute, removed and transferred to modified Leeming Notman agar plate and incubated at 32^0C for 7 days in humid chamber (Guého *et al.* 1996 and Sugita *et al.* 2004). Samples were also collected from forehead, chest, upper back and dorsum of hand of healthy individuals. Samples were collected in duplicate for direct microscopic examination using Parkers's stain, Calcofluor white stain and culture.

Identification Malassezia species

Phenotypic characterization

Malassezia colonies were counted and identified by macroscopic and microscopic features, biochemical tests like catalase, urease and ß glucosidase activity, Tween and Cremophor EL assimilation, growth at 37^0 & 40^0C (Guého *et al.* 1996,1998 and Guillot *et al.* 1996).

Molecular characterization (DNA extraction, PCR-RFLP, sequencing and sequence alignment)

DNA extraction

DNA extraction was performed by Glass bead-phenol-chlorophorm method (Yamada *et al.* 2002).

PCR-RFLP

PCR-RFLP and sequencing of 26SrDNA were standardized using *Malassezia* reference strains from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India (MTCC) and Centraalbureau Schimmelcultures-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands (CBS-KNAW) (Fig. 1).

Amplification was performed using generic primers Mal 1F: 5′-TAACAAGGATTCCCCTAGTA-3′ and Mal 1 R: 5′-ATTACGCCAGCATCCTAAG-3′ (Promega Corporation, Madison, USA) which could yield a PCR product of 580bp (Mirhendi *et al.* 2005; Giusiano *et al.* 2010 and Zia *et al.* 2015). The endonucleases were selected using CLC Sequence Viewer (version 5.0, CLC Bio, Free software) and 26SrDNA sequences of known *Malassezia* obtained from GenBank accession numbers AY743602 (*M. furfur* CBS 1878), AJ249951(*M. globosa* CBS 7966), EF140672 (*M. japonica* CBS 9431), AJ249954 (*M. obtusa* CBS 7876), AJ249953 (*M. sympodialis* CBS 7222), AJ249950 (*M. restricta* CBS 7877), AY743606 (*M. slooffiae* CBS 7956), AJ249952 (*M. pachydermatis* CBS

1879), AB125263 (*M. yamatoensis* CBS 9725), AB070361 (*M. dermatis* CBS 9169), AB075224 (*M. nana* CBS 9557), AJ305330 (*M. equina CBS 9969*), AY743616 (*M. caprae* CBS 10434), GU733708 (*M. cuniculi* CBS 11721) and KM235689 (*M. arunalokei* CBS13387). The sequences were subjected to multiple sequence alignment, followed by in silico restriction digestion by the commonly available enzymes that cut differently in the D1/D2 region of the 26SrDNA genes. The restriction enzymes CfoI and MboI distinctly differentiated all the species of *Malassezia*. The theoretical restriction digestion profile of 26SrDNA of *Malassezia* reference strains was generated and noted using New England BioLabs NEBcutter, version 2.0. available from: http://tools.neb.com/NEBcutter2/) and compared with the RFLP band pattern of the reference *Malassezia* strains (Vincze *et al.* 2003).

Alkaline phosphatase) from Thermo scientific, USA. Eluate was used as purified gene product for sequencing which was performed for both the strands using above-mentioned primers and BigDye® Terminator Cycle Sequencing Kit, Version 3.1 (Applied Biosystems, Foster City, USA). The sequencing reactions were purified and analyzed on ABI 3730 XL Genetic Analyzer (Applied Bio systems® Foster City, USA). The consensus sequences were prepared using BioEdit sequence alignment editor (version 7.2.5) (Hall 1999). The consensus sequences were searched against the GenBank database of the National Center for Biotechnology Information (NCBI) using the BLASTN algorithm (http://blast.ncbi.nlm.nih.gov/). Consensus sequences were also compared with the sequences available in the CBS-KNAW database.

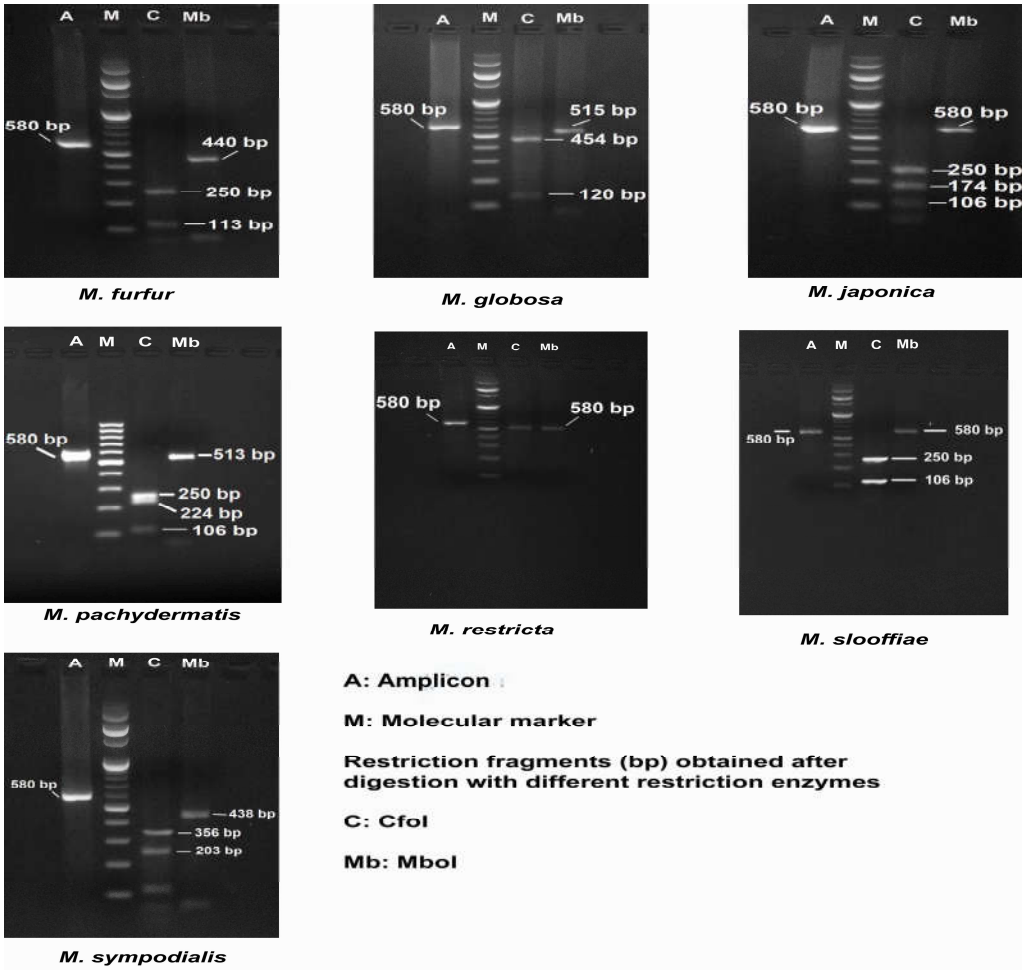

Fig 1 Agarose gel picture of PCR-RFLP profiles of reference strains *M. furfur* (MTCC1374), *M. globosa* (CBS7886), *M. japonica* (CBS 9432), *M. sympodialis* (CBS7222), *M. slooffiae* (CBS7956), *M. restricta* (CBS7877) and *M. pachydermatis* (MTCC1369)

The theoretical restriction fragment size calculated by in silico analysis was compared with gel estimated fragment sizes. Digestion was performed by incubating a 10µl aliquot of amplicon with 10 U of the enzymes in a final reaction volume of 25µl at 37^0C for 3 hours followed by agarose gel electrophoresis. (Table 1; Fig. 2)

Sequencing and sequence alignment

Sequencing of 26S rDNA was done with primers Mal 1F:5′ TAACAAGGATTCCCCTAGTA-3′ and Mal 1 R:5′-ATTACGCCAGCATCCTAAG-3′. Purification of amplified product was performed by Exonuclease I-SAP (Shrimp

A similarity of > 98% to the 26SrDNA partial sequences of type strains was used as criterion for identification of the species.

Statistical analysis of data

Statistical Package for Social Science Software (SPSS version 16.0) and Epi-Info 7.1.2.0 (2013; CDC, Atlanta, USA) were used in the analysis of the data generated in the study. Results are presented as number and percentages or mean/median ± SD. Chi-square test was done to evaluate the correlation between *Malassezia* species and demographic and clinical characteristics. Fisher's exact test was also done to find out

association between various *Malassezia* species and PV. 'Student t test' was done to compare density of the predominant species of the study *M. furfur* in the lesional and non-lesional areas and healthy subjects. The association between predisposing factors and PV was assessed by Chi-square test and logistic regression analysis.

Table 1 PCR product size (bp) and expected length of restriction fragments (bp) obtained in silico restriction digestion profile generated using NEBcutter, version 2.0

Malassezia species	Amplicon size (bp)	CfoI	MboI
M. furfur	582	250,113,107,59,30,21	441,73,68
M. globosa	584	455, 129	516,68
M. obtusa	580	250,153,107,29	440,68,66
M. sympodialis	578	357,200,21	439,73,66
M. restricta	581	NRS	NRS
M. slooffiae	584	250, 107,87,76,64	NRS
M. japonica	580	250,174,107	NRS
M. pachydermatis	580	250,223,107	514
M. yamatoensis	625	165,145,113,85,59	NRS
M. arunalokei	581	430, 151	NRS

NRS: No restriction site

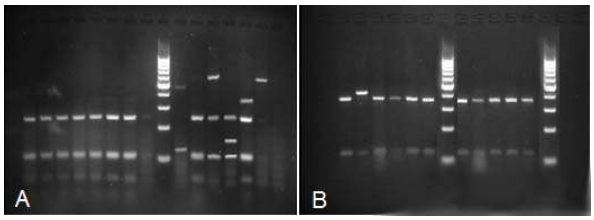

Fig 2 A-PCR-RFLP with CfoI: L1-8-*M. furfur*, L9-100 bp DNA ladder, L10-*M. globosa*, L11-*M. furfur*, L12-*M. restricta & M. furfur*, L13-*M. obtusa*, L-14-*M. furfur and M. sympodialis*, L15-*M. restricta;* B-PCR-RFLP with MboI: L1-*M. furfur*, L2-*M. globosa* L3-6-*M. furfur*, L7-100bp DNA ladder, L8-12-*M. furfur L13-100bp DNA ladder*

Phylogenetic analysis

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Sneath *et al.*, 1973 and Tamura *et al.*, 2013).

RESULTS

Demographic characteristics of the study population

The age of the PV patients ranged from 1 70 years (median: 26 ± 14.6 years) and the number of male cases (median age: 27 ± 14.06 years) was more than females (median age: 27 ± 15.75 years). The PV cases included 135 males (67.5%) and 65 females (32.5%) with a male to female ratio of 1.6:1. PV (36%) was more prevalent in the age group of 21 30 years. Healthy subjects included 86 males (43%) and 114 females (57%). Of the 200 healthy subjects, *Malassezia* was isolated from 105 healthy subjects which included 43 (21.5%) males and 62 (31.0%) females. The highest colonization was seen in the age group of 21 30 years.

Distribution of Malassezia species in PV patients and healthy subjects

The *Malassezia* species isolated from lesional vs non-lesional sites of PV cases were *M. furfur* (72.8% vs 66.1%), *M. globosa* (14.9% vs 4.4%), *M. obtusa* (4.8% vs 0.0%), *M. sympodialis* (3.4% vs 14.7%), *M. restricta* (2.7% vs 19.1%), *M. slooffiae* (0.7% vs 0.0%), *M. japonica* (0.7% vs 0.0%). (Table 2) Among the healthy subjects, the most frequent isolate was *M. furfur* (92.5%). In 7 (5%) PV patients and 2 (1.9%) healthy subjects, mixed growth of more than one *Malassezia* species was seen. *Malassezia* culture positivity rate was higher from the lesional sites (P< 0.0001). (Table 2) The mean colony count was significantly higher in the lesional areas of all the body sites (p value < 0.05). (Table 3)

Table 2 Distribution of *Malassezia* species in PV patients (lesional and non-lesional skin) and healthy subjects

Malassezia species	Lesional n (%)[a]	Non lesional n (%)	Lesional vs non-lesional p-value[b]	Healthy subjects n (%)[a]	Lesional vs Healthy subjects p-value[b]
M. furfur	107 (72.8)[a]	45 (66.1)	0.0126	99 (92.5)[a]	0.0002
M. globosa	22 (14.9)	3 (4.4)	0.0073	3 (2.8)[a]	0.0032
M. obtusa	7 (4.8)	0 (0)	0.0538	0 (0)	0.0225
M. sympodialis	5 (3.4)[a]	10 (14.7)	0.0118	0 (0)	0.0756
M. restricta	4 (2.7)[a]	13 (19.1)	0.0004	5 (4.6)[a]	1.0000
M. slooffiae	1(0.7)	0 (0)	0.0177	0 (0)	1.0000
M. japonica	1 (0.7)	0 (0)	0.0177	0(0)	1.0000
Total *Malassezia* culture positive	140 (70)[c]	68 (34.0)[c]	0.0001	105 (52.5)[d]	0.0005

[a] Patient may have more than one *Malassezia* species
[b] Fisher's exact test: level of significance p < 0.05.
[c] Percentage of 200 PV patients.
d Percentage of 200 healthy subjects.

Comparisons of the results of phenotypic and molecular characterization

Overall some discrepancies were observed when the 26SrDNA PCR-sequencing results were compared with the results of phenotypic characterization and a concordance rate of 74.6% was found. The results of the two molecular methods used in our study were found concordant with a concordance rate of 89.2%.

Correlation with distribution of Malassezia species with demographic and clinical characteristics

Statistical analysis (Chi square test) revealed significant difference between the two genders with reference to distribution of *M. globosa* only in both the study groups. No other *Malassezia* species was significantly associated with any of the demographic and clinical characteristics including involvement of body sites. The results of logistic regression analysis are shown in Table 4. The predisposing factors significantly contributing to development of PV were increased sweating, poor hygeine and immunosuppressive diseases.

Phylogenetic analysis

Phylogenetic tree of the *Malassezia* isolates is shown in Fig. 3. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method. The tree is rooted with *Cryptococcus neoformans* CBS 132 (AF075484). The six *Malassezia* species *M. furfur*, *M. globosa*, *M. obtusa*, *M. sympodialis*, *M. restricta* and *M. japonica*, all formed separate and well-supported clades in the analysis of

Table 3 Mean colony count of *Malassezia furfur* from lesional and non-lesional areas of PV patients

No.	Body site	Mean colony count from lesional area (cfu inch^{-2})	Mean colony count from non-lesional area (cfu inch^{-2})	t-Test for equality of means						
				t	df	*p*-value	Mean difference	Standard error difference	95% CI of the difference	
									Lower	Upper
10	Face	98.7	71.5	3.013	18	0.007*	27.200	9.028	8.231	46.168
28	Neck	107.9	88	3.138	54	0.003*	19.928	6.351	7.195	32.661
25	Chest	90.6	80	2.256	48	0.029*	10.640	4.715	1.158	20.121
19	Back	100.6	80.8	2.829	36	0.008*	19.789	6.996	5.600	33.978
13	Trunk	97.8	71.8	3.277	24	0.003*	26.000	7.934	9.623	42.376
11	Upper limb	82	54.09	8.905	20	0.000*	29.909	3.358	22.902	36.915
4	Lower limb	97	49	4.549	6	0.004*	48.000	10.551	22.181	73.818
1	Flexural area	78	41	-	-	-	-	-	-	-

df: Degree of freedom; CI: Confidence interval
*Statistically significant by Student's t-test (P < 0.05).

Table 4 Logistic regression estimates for the effect of some of the selected variables on development of PV

	Factors		β	Standard Error	*p*	Odd Ratio	95% Confidence Interval for Odd Ratio	
							Lower Bound	Upper Bound
Study group	Occupation	Sedentary Life style (ref.)	-	-	-	1.00	-	-
		Manual Labour	0.317	0.267	0.235	1.373	0.814	2.315
	Application of oil	Yes (ref.)	-	-	-	1.00	-	-
		No	0.064	0.252	0.801	1.066	0.650	1.748
	Increased sweating	Absent (ref.)	-	-	-	1.00	-	-
		Present	1.445	0.224	0.000	4.242	2.734	6.581
	Poor hygiene	Absent (ref.)	-	-	-	1.00	-	-
		Present	0.755	0.317	0.017	2.127	1.142	3.961
	Immuno suppressive disease	Absent (ref.)	-	-	-	1.00	-	-
		Present	2.369	0.616	0.000	10.690	3.194	35.781

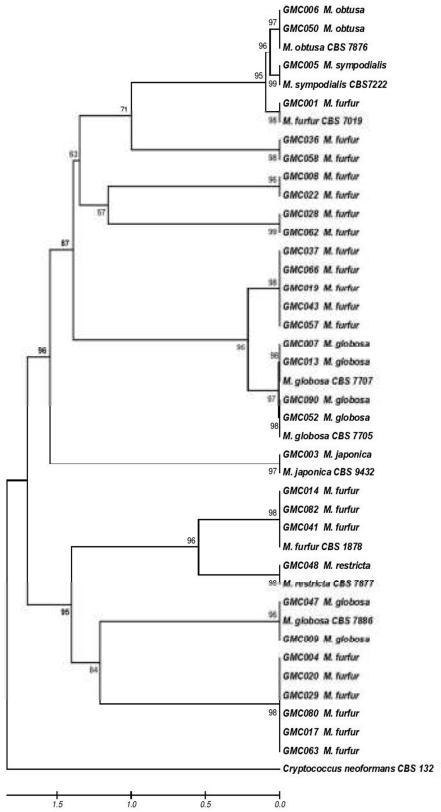

Fig 3 Dendogram showing phylogenetic relationship among the *Malassezia* isolates inferred by UPGMA method using MEGA version 6. The numbers at branch points are the percentages of 1000 bootstrapped datasets that supported the specific internal branches. Outgroup: *Cryptococcus neoformans* CBS 132.

D1/D2 sequences of the 26SrRNA gene of the *Malassezia* species. In this analysis, *M. furfur* forms two well supported clades- a) one with *M. globosa*, *M. obtusa*, *M. japonica* and *M. sympodialis*, and b) the other furfur clade contained *M. furfur*, *M. globosa* and *M. restricta*. Representative sequences of the *Malassezia* isolates were submitted to the NCBI GenBank (accession numbers KT239952 to KT240042).

DISCUSSION

Investigators from different geographical areas have studied the etiology of PV and have observed interesting geographical variations. In most of these studies, *M. globosa* was the predominant species in the PV lesions or species other than *M. globosa* like *M. sympodialis* was the predominant species in the PV lesions (Crespo Erchiga *et al.* 2000; Aspiroz *et al.* 2002; Tarazooie *et al.* 2004; Giusiano *et al.* 2010 and Zhang *et al.* 2013). On the other hand, several studies reported *M. furfur* to be the commonest species associated with PV in the tropical and subtropical regions (Razanakolona *et al.* 2004; de Quinzada 2005; Miranda *et al.* 2006; Krisanty *et al.* 2008; Eidi 2012; Shoeib *et al.* 2013; Pramanik *et al.* 2014 and Ibekwe *et al.* 2015).

In Indian studies also, considerable variations can be found. *Malassezia sympodialis* was reported to be the commonest species isolated from PV lesions in south India; *M. globosa* in north and central India and *M. furfur* was reported to be the commonest species in West Bengal (Panja 1927; Dutta *et al.* 2002; Kindo *et al.* 2004; Choudhury *et al.* 2010 and Pramanik *et al.* 2014). In the present study also, *M. furfur* (72.8%) was the predominant isolate. These findings are similar to the studies done in Madagascar, Panama, Brazil, Indonesia, Iran,

Egypt and Nigeria which show a clear predominance of *M. furfur* in PV lesions (Razanakolona *et al.* 2004; de Quinzada 2005; Miranda *et al.* 2006; Krisanty *et al.* 2008; Eidi 2012; Shoeib *et al.* 2013; Pramanik *et al.* 2014 and Ibekwe *et al.* 2015). This may support the earlier hypothesis proposed by Panja (1927) or Castellani (1935) and later on defended by Midgley (2000) and Crespo-Erchiga and Delgado Florencio (2002) that a second species other than *M. globosa*, which is mainly reported from temperate region, might predominate in warmer and more humid climates (Panja 1927; Castellani 1935; Midgley 2000 and Crespo-Erchiga and Delgado Florencio 2002). In the present study, *M. globosa* has been isolated less frequently (14.9%) than other studies (Roberts 1969; Makni *et al.* 2004 and Tarazooie *et al.* 2004).

The present study revealed that, *Malassezia* species were not significantly associated with any particular age group which was in agreement with the findings of Shokohi *et al.* (2009) and Choudhary *et al.* (2010). However, there was significant difference between the two genders with reference to distribution of *M. globosa* only in both the study groups. This corresponds with the findings of Giusiano *et al.* (2010). The study also found that, none of the *Malassezia* species affected the body sites with statistical significance which corresponds with the findings of Shokohi *et al.* (2009) and Giusiano *et al.* (2010).

CONCLUSION

The results of this study supported the proposed hypothesis that geographical, climatic and racial variations can contribute towards the differences in the distribution of *Malassezia* species. However, in our view, further studies would be needed in this region to understand the relationship of various environmental factors and variation in distribution of *Malassezia* species.

Acknowledgement

I gratefully acknowledge the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, for their financial support in this work under Research & Development project grant vide sanction no.BT/PR3633/MED/29/330/2011 dated 26/03/2011.

I express my gratitude to Dr. Arunaloke Chakrabarti, Professor and Head, Dr. Shivaprakash M Rudramurthy, Professor, Prasanna Honnavar, PhD Scholar, Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India for providing some reference strains.

References

Abou-Gabal M, Chastain CB, Hogle RM. (1979): *Pityrosporum* (*Pachydermatis*) *canis* as a major cause of otitis externa in dogs. *Mycoses*, 22:192-199. http://dx.doi.org/10.1111/j.1439-0507.1979.tb01741.x.

Ashbee HR, Evans EG. (2002): Inmunology of diseases associated with *Malassezia* species. *Clin Microbiol Rev*, 15:21-57. http://dx.doi.org/10.1128/CMR.15.1.21-57.2002.

Aspiroz C, Ara M, Varea M, Rezusta A, Rubio C. (2002): Isolation of *Malassezia globosa* and *M. sympodialis* from patients with pityriasis versicolor in Spain. *Mycopathol,* 154:111-117 http:// dx. doi. org/ 10.1128/CMR.15.1.21-57.2002.

Batra R, Boekhout T, Guého E, Cabaries FJ, Dawson (Jr) TL, Gupta A. (2005): *Malassezia Ballion*, emerging clinical yeasts. *FEMS* Yeast Res, 5:1101 1113. http://dx.doi.org/ 10.1016/j.femsyr.2005.05.006.

Cabanes FJ, Vega S, Castella G. (2011): *Malassezia cuniculi* sp. nov., a novel yeast species isolated from rabbit skin. *Medical Mycology*, 49:40 48. http://dx.doi.org/ 10.3109/13693786.2010.493562.

Cafarichia C, Gasser RB, Figueredo LA, Latrofa MS, Otranto D. (2011): Advances in identification of *Malassezia*. *Mol Cell Probes*, 25:1-7. http://dx.doi.org/10.1016/j.mcp.2010.12.003.

Castellani A. (1935): Pityriasis versicolor tropicalis. *Proc R Soc Med*, 28(12):1558.

Chengappa MM, Maddus RL, Greer SC. (1983): A microbiologic survey of clinically normal and otitic canine ear canals. *Vet Med Small Anim Clin*, 78:343-344.

Choudhury R, Singh S, Banerjee T, Tilak R. (2010): Prevalence of different *Malassezia* species in Pityriasis versicolor in Central India. *Indian J Dermatol Venereol Leprol*, 76:159-164. http://dx.doi.org/ 10.4103/0378-6323.60566.

Crespo Erchiga V, Ojeda MA, Vera Casaño A, Crespo Erchiga A, Sanchez FF. (2000): *Malassezia globosa* as the causative agent of pityriasis versicolor. *Br J Dermatol,* 143: 799-803. http://dx. doi. Org/10. 1046/j.1365-2133.2000.03779.x.

Crespo MJ, Abarca Ml, Caban's FJ. (2000): Atypical lipid-dependent *Malassezia* species isolated from dogs with otitis externa. *J clin Microbiol*, 38:2383-2385.

Crespo-Erchiga V, Delgado Florencio V. (2002): *Malassezia* species in skin diseases. *Curr Opin Infect Dis*, 15:133 142.

Crespo-Erchiga V, Guého E. (2005): Superficial diseases caused by *Malassezia* species. In Hay R and Merz W (eds), Topley and Wilson's Microbiology and Microbial Infections, Mycoloy, 9th ed. Arnold, London, pp. 202 217.

Crespo-Erchiga V, Ojeda Martos A, Vera Casaño A. (1999): Mycology of pityriasis versicolor. *J Mycol Méd,* 9:143-148. http://dx.doi.org/JMM-09-1999-9-3-1156-5233-101019-ART85.

de Quinzada M. (2005): Study of Malassezia species related skin disease, pityriasis versicolor in Panama. PhD thesis, University of Panama.

Dutta S, Bajaj AK, Basu S, Dikshit A. (2002): Pityriasis versicolor: socio-economic and clinicomycologic study in India. *Int J Dermatol*, 41:823-824.

Eichstedt E. (1846): Fungi in the Pityriasis versicolor. *Froriep Neue Notiz Natur Heilk*, 39:270.

Eidi S, (2012): Biochemical characterizations and Genotyping by RAPD-PCR analyses of *Malassezia* spp. from pityriasis versicolor and seborrhoeic dermatitis patients. *Iranian Red Crescent Med J,* 14:710-715. http://dx.doi.org/10.5812/ircmj.3815.

Giusiano G, Sosa MDLA, Rojas F, Vanacore ST, Mangiaterra M. (2010): Prevalence of *Malassezia* species in pityriasis versicolor lesions in northeast Argentina. *Rev Ilberoam Micol*, 27:71 74. http://dx.doi.org/10.1016/j.riam.2009.12.005.

Guého E, Boekhout T, Ashbee HR, Guillot J, van Belkum A, Faegermann J. (1998): The role of *Malassezia* species in the ecology of human skin and as pathogens. *Med Mycol*, 36:220-229.

Guého E, Midgley G, Guillot J. (1996): The genus *Malassezia* with description of four new species. *Antonie Leeuwenhoek*, 69:337-355. http:// dx. doi. Org /10.1007/BF00399623.

Guillot J, Gue´ho E, Lesourd M, Midgley G, Chevrier G, Dupont B (1996): Identification of *Malassezia* species. A practical approach. *J Mycol Med*, 6:103–110.

Gupta AK, Kohli Y, Faergemann J, Summerbell RC. (2001): Epidemiology of the *Malassezia* yeast associated with pityriasis versicolor in Ontario, *Canada. Med Mycol*, 39:199-206. http: //dx. doi. Org / 10. 1080 / mmy. 39. 2. 199.206.

Gupta AK, Yatika K, Summerbell RC. (2000): Molecular differentiation of seven *Malassezia* species. *J Clin Microbiol,* 38:1869-1875.

Gupta P, Chakrabarti A, Singhi S, Kumar P, Honnavar P, Shivaprakash MR. (2014): Skin Colonization by *Malassezia* spp. in hospitalized neonates and infants in a tertiary care centre in North India. *Mycopathol*, 178:267-272. http://dx.doi.org/10.1007/s11046-014-9788-7.

Hall TA. (1999): BioEdit: a user friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucl. Acids,* 41:95-98.

Honnavar P, Prasad GS, Ghosh A, Dogra S, Handa S and Rudramurthy SM. (2016): *Malassezia arunalokei* sp. nov., a novel yeast species isolated from seborrhoeic dermatitis patients and healthy individuals from India. *J Clin Microbiol*, 54:1826 1834. http:// dx. doi. org/ 10. 1128/JCM.00683-16.

Ibekwe PU, Ogunbiyi AO, Besch R, T. Ruzicka T, Sárdy M. (2015): The spectrum of *Malassezia* species isolated from students with pityriasis vesicolor in Nigeria. *Mycoses*, 58:203-208. http://dx.doi.org/10.1111/ myc.12298.

Kindo AJ, Sophia SKC, Kalyani J, Anandan S. (2004): Identification of *Malassezia* species. *Indian J Med Microbiol*, 22:179-181.

Krisanty RIA, Bramono K, Made Wisnu I. (2008): Identification of *Malassezia* species from pityriasis versicolor in Indonesia and its relationship with clinical characteristics. *Mycoses*, 52:257-262. http://dx.doi.org/ 10.1111/j.1439-0507.2008.01593.x.

Makni F, Ben Salah I, Affes M, Cheikhrouhou F, Sellami A, Sellami H, *et al*. (2004): Identification of *Malassezia* species isolated from pityriasis versicolor lesions and healthy subjects in the region of Sfax (Tunisie) (Abstract). *FSMM J Mycol Méd*, 14:159.

Midgley G. (2000): The lipophilic yeast: state of the art and prospect. *Med Mycol*, 38:9-16.

Miranda KC, de Araujo CR, Soares AJ, de Aquino Lemos J, Souza LK, do Rosário Rodrigues Silva M. (2006): Identification of *Malassezia* species in patients with pityriasis versicolor in Goiânia-GO. *Rev Soc Bras Med Trop*, 39:582-583.

Mirhendi H, Makimura K, Zomorodian K, Yamada T, Sugita T, Yamaguchi H. (2005): A simple PCR-RFLP method for identification and differentiation of 11 *Malassezia* species. *J Microbiol Methods*, 61:281 284. http:// dx. doi.org/ 10.1590/S0037-86822006000600017.

Moore RT. (1980): Taxonomic proposals for the classification of marine yeasts and other yeasts-like fungi including the smuts. *Bot Mar*, 23(6):361–373.

Nakabayashi A, Sei Y, Guillot J. (2000): Identification of *Malassezia* species isolated from patients with seborrhoeic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. *Med Mycol*, 38:337-341. http://dx.doi.org/10.1080/mmy.38.5.337.341.

Panja G. (1927): The *Malassezia* of the skin, their cultivation, morphology and species. *Trans Th Congs Far Easthern Ass Trop Med*, 2:442-456.

Pramanik SB, Bandopadhyay M and Nandi A. (2014): A study of prevalence of different species of *Malassezia* causing pityriasis versicolor in different age groups in a tertiary care hospital in Kolkata. *IOSR-JDMS*, 13:88-92.

Raabe P, Mayser P, Weiss R. (1998): Demonstration of *Malassezia furfur* and *M. sympodialis* together with *M. pachydermatis* in veterinary specimens. *Mycoses*, 41:493-500.http://dx.doi.org/10.1111/j.1439-0507. 1998. tb00712.x.

Razanakolona I, Rakotozandrindrainy N, Razafimahefa J, Andriatsilavo T, Grosjean P, Contet-Audonneau N. (2004): Pityriasis versicolor á Antananarivo: premiére etude sur I identification d´ espéces *Malassezia* responsables (Abstract). *FSMM J Mycol Méd*, 14:152.

Roberts SOB. (1969): Pityriasis versicolor: a clinical and mycological investigation. *Br J Dermatol*, 81:315-326. http://dx.doi.org/10.1111/j.1365-2133.1969.tb13990.x.

Shoeib MA, Gaber MA, Labeeb AZ, El-Kholy OA. (2013): *Malassezia* species isolated from lesional and nonlesional skin in patients with pityriasis versicolor. *Menoufia Medical Journal*, 26:86-90. http://dx.doi.org/ 10.1111/j.1439-0507.2006.01310.x.

Sneath PHA, Sokal RR. (1973): Numerical Taxonomy. Freeman, San Francisco.

Sugita T, Tajima M, Takashima M, Amaya M, Saito M, Tsuboi R, *et al*. (2004*):* A new yeast, *Malassezia yamatoensis*, isolated from patient with seborrhoeic dermatitis, and its distribution in patients and healthy subjects. *Microbiol Immunol*, 48:579-583.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. (2013): MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30:2725-2729. http://dx.doi.org/10.1093/molbev/mst197.

Tarazooie B, Kordbacheh P, Zaini F, Zomorodian K, Saadat F, Zeraati H *et al*. (2004): Study of the distribution of *Malassezia* species in patients with pityriasis versicolor and healthy individuals in Tehran, Iran. *BMC Dermatol*, 4:5. http://dx.doi.org/10.1186/1471-5945-4-5.

Vincze T, Posfai J, Roberts RJ. (2003): NEB cutter: a program to cleave DNA with restriction enzymes. *Nucleic Acids Res*, 31:3688-3691.

Yamada Y, Makimura K, Mirhendi H, Ueda K, Nishiyama Y, Yamaguchi H, *et al*. (2002): Comparison of different methods for extraction of mitochondrial DNA from human pathogenic yeasts. *Jpn J Infect Dis*, 55:122-125.

Zhang H, Ran Y, Xie Z, Zhang R. (2013): Identification of *Malassezia* Species in Patients with Seborrheic Dermatitis in China. *Mycopathol*, 175:83-89. http://dx.doi.org/10.1007/s11046-012-9606-z.

Zia M, Mirhendi H, Toghyani M. (2015): Detection and identification of *Malassezia* species in domestic animals and aquatic birds by PCR-RFLP. *Iranian Journal of Veterinary Research*, 16: 36-41.

How to cite this article:

Sharma, A *et al*.2017, Characterization of Malassezia Species Isolated From Pityriasis Versicolor Patients And Healthy Subjects of North-East India By PCR-RFLP And 26SrDNA Sequencing. *Int J Recent Sci Res.* 8(4), pp. 16624-16631.
DOI: http://dx.doi.org/10.24327/ijrsr.2017.0804.0186

****