International Journal of Systematic Bacteriology (1999), **49**, 1287–1294 Printed in Great Britain

Genetic diversity in the yeast species *Malassezia pachydermatis* analysed by multilocus enzyme electrophoresis

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Fifty-two strains of the yeast species *Malassezia pachydermatis* were analysed by multilocus enzyme electrophoresis. *M. pachydermatis* appeared to be genetically heterogeneous. A total of 27 electrophoretic types were identified that could be divided into five distinct groups with different host specificities. The diversity revealed by this electrophoretic method matched remarkably well the reported genetic variability obtained by comparing large subunit rRNA sequences. This study also suggests that genetic exchanges can occur in the anamorphic species *M. pachydermatis*.

Keywords: yeast, multilocus enzyme electrophoresis, rDNA sequence analysis, genetic diversity, host

INTRODUCTION

The lipophilic yeasts belonging to the genus *Malassezia* Baillon are all small cells that undergo unilateral and repetitive budding (Yarrow & Ahearn, 1984). These yeasts are part of the normal cutaneous microflora of warm-blooded vertebrates. They have been reported to cause, under special conditions, a range of cutaneous diseases in humans (Marcon & Powell, 1992) and in animals, especially pet carnivores (Scott *et al.*, 1995). Human systemic infections (Redline *et al.*, 1985; Welbel *et al.*, 1994) have also been described, especially in patients on parenteral feeding supplemented with lipids.

The genus *Malassezia* contained only two species for many years (Yarrow & Ahearn, 1984), *Malassezia furfur*, a lipophilic yeast that requires long-chain fatty acids for growth (lipid-dependent species) (Nazzaro Porro *et al.*, 1976) and *Malassezia pachydermatis*, which can take advantage of the short-chain fatty acids present in basic mycological media such as Sabouraud glucose agar (non-lipid-dependent species) (Ahearn & Simmons, 1998; Slooff, 1970; Yarrow & Ahearn, 1984). However, there was no consensus about this limited number of species. Some authors demonstrated clearly that *M. furfur* was a polymorphic species. Midgley (1989, 1993) identified two groups of lipid-dependent yeasts on morphological, physiological and immunological grounds. Cunningham *et al.* (1990) also showed that *M. furfur* could be subdivided into three serovars on the basis of group-specific surface antigens. Simmons & Guého (1990) described

Abbreviations: AAT, alanine aminotransferase; ET, electrophoretic type; FCA, factorial correspondence analysis; FUM, fumarase; GPI, glucose-phosphate isomerase; HK, hexokinase; IDH, isocitrate dehydrogenase; LAP, leucine aminopeptidase; LSU, large subunit; MLEE, multilocus enzyme electrophoresis; MPI, mannose-phosphate isomerase; NP, purine-nucleoside phosphorylase; PEP1, peptidase 1; PEP3, peptidase 3; PGD, phosphogluconate dehydrogenase; PGM, phosphoglucomutase; SDH, sorbitol dehydrogenase.

Table 1. Source and electrophoretic type of the 52 *M. pachydermatis* isolates analysed

Strain numbers were designated by E. Guého (EG); G. Midgley, St John's Institute of Dermatology, London, UK (GM); Centraalbureau voor Schimmelcultures, Delft, The Netherlands (CBS); J. Guillot (JG); J. Nicolet, University of Bern, Switzerland (JN); R. Bond, Royal Veterinary College, University of London, UK (RB); and R. Vanbreuseghem, Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium (RV).

Host	Isolate	Site	Country	ET
Primates				
Human	EG 601	Septicaemia	USA	1
Human	EG 610	Sputum	USA	1
Human	GM 401	Healthy skin	USA	1
Human	GM 407	Septicaemia	USA	1
Human	GM 437	Healthy skin	UK	1
Human	GM 468	Healthy skin	UK	1
Human	EG 680	Vaginitis	France	5
Monkey	GM ZM46	Healthy skin	UK	1
Gorilla	JG 566	Healthy ear	France	14
Carnivores				
Dog	CBS 1879^{NT}	Otitis externa	Sweden	1
Dog	GM 458	Healthy back	UK	1
Dog	JG 18E	Otitis externa	France	1
Dog	JG 44B	Healthy back	France	1
Dog	JG 44E	Otitis externa	France	1
Dog	JG 45	Skin lesion	France	1
Dog	JN 447	Otitis externa	Switzerland	1
Dog	RB 1	Healthy skin	UK	1
Dog	JG 16	Healthy back	France	3
Dog	JG 30	Healthy ear	France	14
Dog	GM 440	Healthy back	UK	17
Dog	GM 456	Healthy back	UK	18
Dog	JG 42	Skin lesion	France	18
Dog	GM 469	Healthy back	UK	19
Dog	GM 443	Healthy back	UK	20
Dog	JN 894	Otitis externa	Switzerland	21
Dog	RB 6	Healthy mouth	UK	22
Dog	JG 111	Otitis externa	France	23
Dog	JG 1B	Skin lesion	France	25
Dog	RB 3	Healthy anus	UK	26
Dog	RB 4	Healthy ear	UK	27
Cat	GM 435	Healthy back	UK	1
Cat	JG 508	Skin lesion	France	3
Cat	JG 518B	Healthy back	France	4
Cat	JG 527	Healthy ear	France	7
Cat	JG 511	Healthy back	France	16
Cat	JG 509	Skin lesion	France	17
Tiger	JG 581	Healthy back	France	1
Fox	GM ZC2	Healthy skin	UK	1
Tayra	GM ZF3	Healthy skin	UK	1
Civet	GM 439	Healthy skin	UK	2
Fennec fox	JG 562	Otitis externa	France	3
Brown bear	JG 560	Healthy ear	France	6
Ferret	JG 561	Otitis externa	France	8
Seal	JG 564	Healthy ear	France	10
Herbivores				
Wallaby	JG 565	Healthy back	France	11
White rhinoceros	JG 576B	Healthy back	France	1
White rhinoceros	RV 70583	Skin lesion	Belgium	12
White rhinoceros	JG 577	Healthy ear	France	12
White rhinoceros	JG 578	Healthy back	France	13
Rabbit	JG 574	Healthy ear	France	15
Horse	JG 550	Healthy ear	France	24
Other				
Pig	GM 442	Healthy skin	UK	9

CBS 1879^{NT} is the neotype strain of *M. pachydermatis* (type strain of *Pityrosporum canis*).

a new lipid-dependent species named *Malassezia sympodialis*, based on its alternative type of budding (Ahearn & Simmons, 1998). Boekhout & Bosboom (1994) demonstrated that four different karyotypes could be displayed by the lipid-dependent strains. The diversity of these yeasts was clarified definitively by comparison of the large subunit (LSU) rRNA sequences (Guillot & Guého, 1995). This comparison led Guého *et al*. (1996) to divide the lipid-dependent yeasts into six taxa, including *M. furfur* and *M. sympodialis* and four new species, *Malassezia globosa*, *Malassezia obtusa*, *Malassezia restricta* and *Malassezia slooffiae*.

The taxonomic position of the only non-lipid-dependent species, *M. pachydermatis*, was less controversial. However, the two hypervariable regions, D_1 and D_2, of the LSU indicated genetic diversity within this species. Sequencing of rRNA from a large number of *M. pachydermatis* strains, mainly isolated from animals but also from humans, allowed the species to be divided into seven sequence types (sequevars Ia–Ig) (Guillot & Guého, 1995; Guillot *et al*., 1997).

The diversity observed as a result of rRNA sequencing is now compared with that detected by another technique that is also very informative. Multilocus enzyme electrophoresis (MLEE) has been used as a standard method for examining the genetics of eukaryotic populations (Ayala, 1976; Lewicka *et al*., 1995; Lewontin, 1974; Nevo *et al*., 1980, Pujol *et al*., 1993; Selander & Whittam, 1983) and systematics (Holzschu *et al*., 1983; Oxford & Rollingon, 1983). It was therefore used to obtain a second picture of the genetic diversity of *M. pachydermatis*. This paper describes the analysis of multilocus genotypes in the 52 strains of *M. pachydermatis* that were previously characterized by LSU rRNA sequencing.

METHODS

Yeast strains. The 52 strains of *M. pachydermatis* examined are listed in Table 1 with their original identification number, host site of isolation and geographical origin. They were obtained from the Mycological Unit of the Pasteur Institute (Paris, France), where they are maintained in a collection. All the strains were sequenced by Guillot & Guého (1995) and Guillot *et al*. (1995, 1997). A maximum sequence divergence of five differences (2 % base substitution) was observed in the hypervariable region D_2 of LSU rRNA. However, in spite of these differences, all strains were considered to be of the single species *M. pachydermatis*, because of the high DNA–DNA reassociation values, higher than 80 % in all cases (Guillot & Guého, 1995; Guillot *et al*., 1995).

Enzyme extracts. All yeast cells were cultured in Roux flasks on 166 ml Sabouraud 0·05 % chloramphenicol/0·05 % cycloheximide/glucose agar, at 34 °C for 72 h. Cells were harvested by centrifugation at 1000 ***g*** for 5 min and were suspended in 5 ml distilled water. Yeast cells were disrupted in a Braun cell homogenizer with 0·25 mm diameter glass beads for 1 min. The mixture was then centrifuged at 12000 ***g*** and 4 °C for 30 min. The supernatant of each isolate was distributed into 300 ml aliquots and stored at −20 °C.

Enzyme electrophoresis. Starch gel electrophoresis and specific enzymic staining were performed according to published protocols (Ben Abderrazak *et al*., 1993; Pasteur *et al*., 1987; Richardson *et al*., 1986; Shaw & Prasad, 1970) with a few small modifications. A total of 13 enzymic systems were analysed: aspartate aminotransferase (AAT; EC 2.6.1.1), fumarase (FUM; EC 4.2.1.2), glucose-phosphate isomerase (GPI; EC 5.3.1.9), hexokinase (HK; EC 2.7.1.1), isocitrate dehydrogenase (IDH; EC 1.1.1.42), leucine aminopeptidase (LAP; EC 3.4.11.1), mannose-phosphate isomerase (MPI; EC 5.3.1.8), purine-nucleoside phosphorylase (NP; EC 2.4.2.1), peptidase 1 (PEP1; EC 3.4.11; substrate, Val–Leu), peptidase 3 (PEP3; EC 3.4.11; substrate, Lys–Leu), phosphogluconate dehydrogenase (PGD; EC 1.1.1.43), phosphoglucomutase (PGM; EC 5.4.2.2) and sorbitol dehydrogenase (SDH; EC 1.1.1.14). Alleles were numbered in increasing order of anodal mobility. Each isolate was characterized by its allelic combination at the various polymorphic enzymic loci. Distinctive multilocus variants were designated electrophoretic types (ETs).

Analysis of the genetic relationships between ETs. The electrophoretic diversity of *Malassezia* isolates was analysed by factorial correspondence analysis (FCA) (Benzecri, 1982; Lebart *et al*., 1984) with the PRAXIS-PC software, version 2.0 (Praxeme R & D, Biométrie, Centre National de la Recherche Scientifique, Montpellier, France). FCA was performed using a contingency table (isolates × alleles) in which each isolate was represented by its allelic makeup (She *et al*., 1987). Each isolate was described for each allele by the values 2, 1 or 0 according to whether it possessed either two (homozygote), one (heterozygote) or no copies of the allele in question. This method of analysis characterized each isolate in terms of all the genetic variables (alleles) and showed the contribution of each allele to the overall differences between the isolates (Coustau *et al*., 1991). Nei's genetic distances (Nei, 1978) were calculated with the GENETIX-PC software, version 3-3 (Belkhir *et al*., 1996). The relationships between ETs were visualized by using a dendrogram constructed by the neighbour-joining method (Saitou & Nei, 1987) with the NJTREE-PC software, version 2.0. This method finds pairs of operational taxonomic units (neighbours) that minimize the total branch length at each stage of clustering operational taxonomic units, starting with a star-like tree.

RESULTS

Table 2 shows the allelic distribution observed and reveals a strong genetic diversity among the isolates analysed. The 13 enzymic loci examined were polymorphic and displayed a total of 44 alleles. The number of alleles per locus ranged from two to six. This genetic diversity yielded up to 27 different ETs. Six ETs were represented more than once: 20 isolates belonged to ET1; three isolates belonged to ET3 and two isolates belonged to ET12, ET14, ET17 and ET18. Thus, 21 of the 52 isolates studied had a specific genotypic makeup (Table 2). Table 1 shows that ETs were encountered in various host species and geographical areas. For example, ET1 was isolated from a human in the USA, from dogs in Sweden, the UK, France and Switzerland, from cats and foxes in the UK and from a rhinoceros and a tiger in France (zoological gardens).

Table 2. Allelic patterns observed at 13 putative enzymic loci for 27 ETs of the 52 isolates of *M. pachydermatis* analysed

Alleles were numbered in increasing order of anodal mobility. Characteristic alleles of the different groups of ETs are indicated. NA, Not available.

Isolate	ET (sequevar)	Alleles observed at putative enzyme loci													Characteristic alleles
		AAT	FUM	GPI	HK	IDH	LAP	MPI	NP	PEP1	PEP3	PGD	PGM	SDH	
Group I															
CBS 1879^{NT}	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
EG 601	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
EG 610	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
GM 401	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
GM 407	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
GM 435	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
GM 437	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
GM 458	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
GM 468	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
GM ZC2	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
GM ZF3	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
GM ZM46	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
JG 18E	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
JG 44B	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
JG 44E	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	GPI-1, PGD-3
JG 45	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
JG 576B	1 (Ic)	3	2	1	3	3	2	2	2	2	2	3	2	2	
JG 581	1 (Ia)	3	2	1	3	3	2	2	2	2	2	3	2	2	
JN 447	1 (Id)	3	2	1	3	3	2	2	2	2	2	3	2	2	
RB 1	1 (Id)	3	2	1	3	3	2	2	2	2	2	3	2	2	
GM 439	2 (Ia)	3	2	1	3	3	2	2	1	2	2	2	2	2	
JG 16	3 (Ia)	3	2	1	3	3	2	2	2	2	2	2	2	2	
JG 508	3 (Ia)	3	2	1	3	3	2	2	2	2	2	2	2	2	
JG 562	3 (Ia)	3	2	1	3	3	2	2	2	2	2	2	2	2	
JG 518B	4 (Ib)	3	2	1	3	3	2	2	2	2	1	2	2	2	
EG 680	5 (Ia)	3	2	1	3	3	2	2	2	2	1	3	2	2	
JG 560	6 (Ib)	3	2	1	3	3	2	2	1	2	1	2	2	2	
JG 527	7 (Ia)	3	1	1	3	3	2	2	2	2	3	2	1	2	
JG 561	8 (Ig)	2/3	2	1/3	3	3	1/2	2	2	2	2	4	1/2	2	
GM 442	9 (If)	3	3	2	3	2	1	1	2	2	4	3	1	2	
JG 564	10 (Ib)	3	2	3	2	2	1	2	3	1	2	2	2	2	IDH-2, HK-2,
JG 565	11 (Ib)	3	2	3	2	2	1	2	3	1	2	2	1	2	NP-3
JG 577	12 (Ic)	3	3	3	4	1	1	2	4	2	4	1	2	3	IDH-1, HK-4,
RV 70583	12 (Ic)	3	3	3	4	1	1	2	4	2	4	1	2	3	PGD-1
JG 578	13 (Ic)	3	3	3	4	1	1	2	4	2	4	1/3	2	3	
Group II															
JG 30	14 (Ie)	2	3	2	1	3	1	1	4	1	2	2	3	2	
JG 566	14 (Ie)	2	3	2	1	3	1	1	4	1	2	2	3	2	
JG 574	15 (Ie)	2	3	2	1	3	1	1	2	1	2	2	2	1	
JG 511	16 (Ie)	2	3	2	1	3	1	1	4	1	2	2	2	2	AAT-2, GPI-2,
GM 440	17 (Ie)	2	3	2	1	3	1	1	4	1	2	2	2	1	HK-1
JG 509	17 (Ie)	2	3	2	1	3	1	1	4	1	2	2	2	1	
GM 456	18 (Ie)	1	3	2	1	3	1	1	4	1	2	2	2	2	
JG 42	18 (Ie)	1	3	2	1	3	1	1	4	1	2	2	2	2	
Group IIIA															
GM 469	19 (Id)	3	3	4	3	3	2	3	2	1	4	2	1	3	
GM 443	20 (Id)	3	2	6	4	3	2	2	2	1	4	2	1	3	GPI-4, GPI-5,
JN 894	21 (Id)	3	3	6	3	3	2	1	2	1	4	2	1	3	GPI-6, PGM-1
RB 6	22 (Id)	3	2/3	5	3	3	2	3	2	1	4	2	1	3	
Group IIIB															
JG 111	23 (Ie)	2/3	3	2/6	3	3	2	1	4	1	2/3	2	2	2	
JG 550	24 (Ie)	2/3	3	2/6	3	3	2	1	2	1	2/3	2	2	2	
JG 1B	25 (Id)	2/3	2/3	2/6	3	3	2	1	2	1	2/4	2	1	3	PEP3-3
RB 3	26 (Id)	2/3	3	2/6	3	3	2	3	NA	1	2/3	2	1/2	2	
RB 4	27 (Id)	2/3	3	2/6	4	3	NA	3	2	1	2/3	2	1/2	NA	

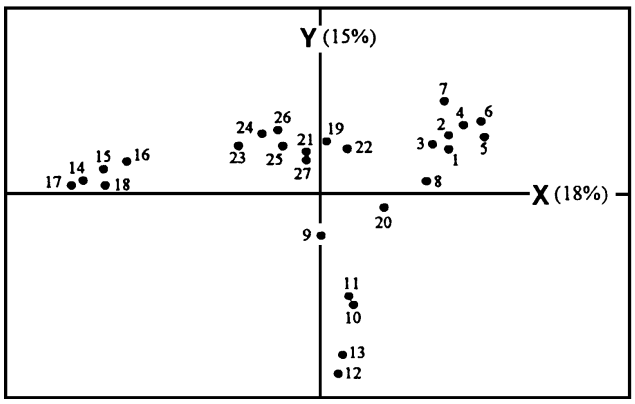

Fig. 1. First plane projection of FCA in two informative axes (X, Y) on the 27 ETs. The contribution of axes to the overall genetic variability is shown in parentheses.

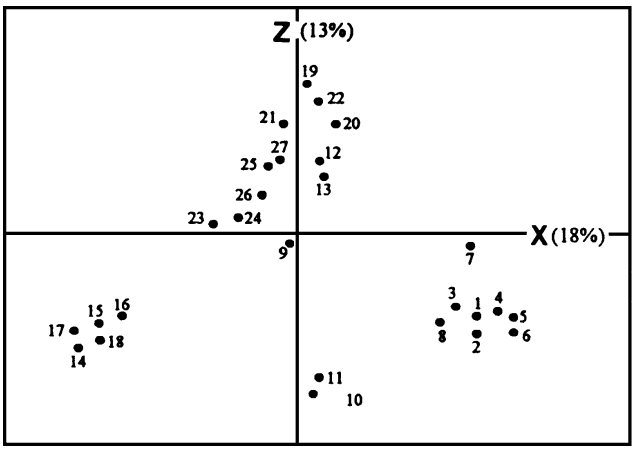

Fig. 2. Second plane projection of FCA in two informative axes (X, Z) on the 27 ETs. The contribution of axes to the overall genetic variability is shown in parentheses.

Seven loci showed phenotypic patterns characteristic of genotypic heterozygotes. However, the heterozygotes were not distributed randomly among the various isolates and loci. Only eight (JG 111, JG 1B, JG 550, JG 561, JG 578, RB 3, RB 4 and RB 6) of the 52 isolates investigated had heterozygote genotypes and all of these except isolates RB 6 and JG 578 were heterozygotes for at least three loci (Table 2).

FCA revealed inter-group differentiation based on the 43 active allelic variables. Three major groups were identified from the projection of isolates along the three most informative axes (X, Y, Z) (Figs 1 and 2). They represented 46% (18+15+13%) of the overall variability. Group I was composed of eight ETs (ET1–ET8), group II contained five ETs (ET14–ET18) and group III included nine ETs (ET19–ET27). Five other ETs were excluded from these three main groups; ET9 was distinct from all the others; ET10 and ET11 seemed to be related and distinct from all the others, as were ET12 and ET13. Table 2 shows the alleles defining the three groups and the other isolates.

Because the majority of heterozygote isolates were in group III, this group was divided into sub-groups IIIA and IIIB; the latter included the isolates that were heterozygotic at three or more loci. This differentiation resulted mainly from the presence of different alleles at loci AAT, GPI, PEP3, PGM and SDH.

Fig. 3 shows that the phylogenetic analysis inferred from MLEE agrees with that based on the LSU rRNA sequence comparisons (Guillot & Guého, 1995). The figure also confirms the position of sub-group IIIB (ETs 23, 24, 26 and 27) between group II (ETs 14–18) and sub-group IIIA (ETs 19–22 and 25).

DISCUSSION

MLEE revealed considerable genetic variation within the species *M. pachydermatis*, leading to its partition into six genetic groups: (i) three major groups (I, II and III containing eight, five and nine ETs, respectively) and (ii) three minor groups (ET9; ET10 and ET11; ET12 and ET13) (Table 2). This variability was previously suggested by LSU rRNA sequencing (Guillot & Guého, 1995; Guillot *et al.*, 1997), which led to *M. pachydermatis* being divided into seven groups defined by small differences in rRNA sequences (sequevars Ia–Ig) (Guillot & Guého, 1995). There is good correspondence between the groups defined by MLEE and those established by rRNA sequencing (Table 2 and Fig. 3). The exceptions are six isolates, JG 576B (sequevar Ic), JN 447 and RB 1 (sequevar Id), JG 562 and JG 560 (sequevar Ib), and JG 561 (sequevar Ig). Group I contains principally sequevar Ia isolates, group II corresponds to the single sequevar Ie, group IIIA to sequevar Id and group IIIB to sequevar Id or Ie. ET9 corresponds to sequevar If, ETs 10 and 11 to sequevar Ib and ETs 12 and 13 to sequevar Ic. The heterogeneity of *M. pachydermatis* revealed by MLEE confirms the results obtained by other methods. Bond & Anthony (1995) described isolates of *M. pachydermatis* with small colonies that were more markedly lipid-dependent than isolates with large colonies. All isolates with small colonies and marked nutritional requirements were found to belong to sequevar Id (Guillot & Guého, 1995). The same morphological diversity of *M. pachydermatis*, with two distinct colony types, was pointed out by Huang *et al.* (1993) and Kiss *et al.* (1996). Huang *et al.* (1993) also found a few biochemical differences in carbohydrate assimilation. More recently, the heterogeneity of *M. pachydermatis* was demonstrated by Coutinho & Rodrigues Paula (1997) using the yeast killer system. They found eight biotypes among 30 isolates from dogs, three of them representing 75% of the isolates whatever their location on the host. These results were obtained with isolates from dogs as the sole host and more differences are likely to be revealed when the survey is extended to other animals. The heterogeneity of *M. pachydermatis* revealed by MLEE and rRNA

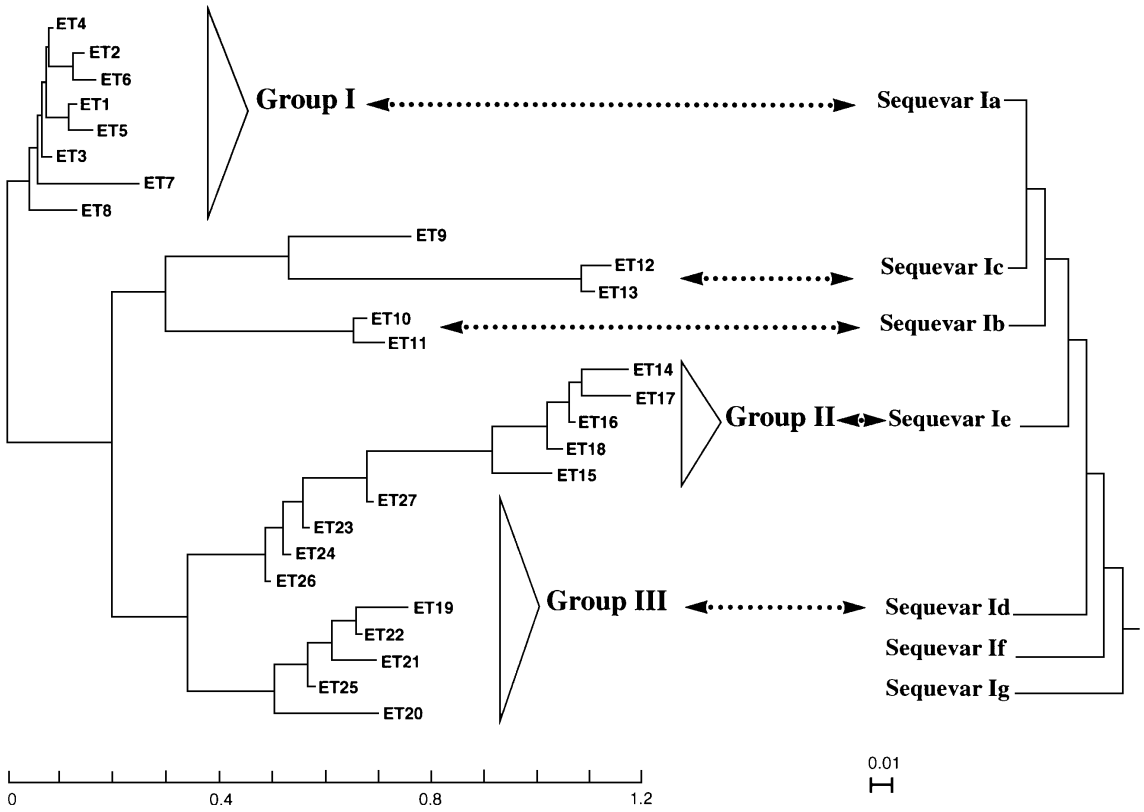

Fig. 3. Genetic relationships among *M. pachydermatis* isolates inferred from MLEE analysis (left) and partial LSU rRNA sequence comparison (right). Dendrograms were created by the neighbour-joining method (see Methods). ETs and sequence-type designations (sequevars) are shown in Table 2. Scale bars represent Nei's genetic distances (left) and accumulated differences per 100 nucleotides (right).

sequencing thus confirms the morphological and physiological differences reported previously. These results suggest that the species *M. pachydermatis* is not a unique genetic entity, but is composed of several genetic groups that remain to be defined clearly in spite of the fact that all isolates could be maintained as a single species by DNA–DNA reassociation experiments (Guillot & Guého, 1995; Guillot *et al.*, 1995, 1997; Guého *et al.*, 1996). These groups could be discrete typing units, as recently defined by Tibayrenc (1998).

The results of MLEE also suggest that there is a correlation between ETs and host specificity. For instance, all the isolates recovered from humans belonged to group I and the sequence Ia, as demonstrated by 25S rRNA analysis (Guillot & Guého, 1995). ET12 and ET13 were similarly recovered only from rhinoceros. Group IIIA and IIIB were recovered mainly from dogs. However, isolates from dogs also appeared in groups I and II. Unlike host specificity, there is no evidence of any link between ETs and geographical origin. However, many more isolates for all sources (host as well as geography) must be examined in order to obtain a clear picture of both correlations. The particular distribution of genotypic patterns observed led us to consider the genetic structures of the isolates analysed. The heterozygotes were not distributed randomly within groups, since the great majority of them were encountered in group IIIB, which contained five ETs. The heterozygotic ETs in this group were also polymorphic at at least three loci. The group IIIB isolates had genotypes intermediate between those in group II and group IIIA (Fig. 2 and Table 2). Group IIIB also had an intermediate pattern as far as LSU rRNA sequencing was concerned. The group II isolates belonged to sequevar Ie, those in group IIIA belonged to sequevar Id and those in group IIIB had sequevars Ie or Id. It is evident that the congruence of results obtained from two independent sets of data is not due to chance alone.

Although we cannot specify how these organisms reproduce, these results suggest strongly that genetic exchange occurs within and/or between the various genetic groups of *M. pachydermatis*. Many other eukaryotic micro-organisms are assumed to reproduce sexually, usually from analysis of phenotype frequency distributions, rather than on the direct observation of teleomorphic features (Tibayrenc *et al.*, 1991). The occurrence of more than one genetic type (sequevar) on the skin of a host individual (Guillot *et al.*, 1997) reinforced the possibility of sexuality in *M.*

pachydermatis. A similar conclusion was proposed by Mittag (1994) for the generic type species *M. furfur*, by using two strains CBS 1878 and CBS 6001 which were shown to belong to this species by genome comparison (Boekhout *et al*., 1998). Variations in the cell surface/volume quotient suggested that a population of *Malassezia* yeasts could be a mixture of cells with different genomes (haploid, diploid and maybe polyploid). When cultured on a medium that induced filamentation, the *M. furfur* isolate CBS 6001 showed ultrastructural features that Mittag *et al*. (1994) compared to a teleomorphic feature (metabasidium) of other basidiomycetous yeasts (Ustilaginales). These results indicate that yeasts of the anamorphic genus *Malassezia* in fact have a teleomorphic state, a conclusion of particular importance for the epidemiology and pathology of these opportunistic fungi.

REFERENCES

Ahearn, D. G. & Simmons, R. B. (1998). *Malassezia* Baillon. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 782–784. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.

Ayala, F. J. (1976). *Molecular Evolution*. Sunderland, MA: Sinauer.

Belkhir, K., Borsa, P., Goudet, J., Chickhi, L. & Bonhomme, F. (1996). *Logiciel sous Windows TM pour la génétique des populations*. CNRS UPR 9060, Université de Montpellier II, Montpellier, France.

Ben Abderrazak, S., Guerrini, F., Mathieu-Daudé, F., Truc, P., Neubauer, K., Lewicka, K., Barnabé, C. & Tibayrenc, M. (1993). Isoenzyme electrophoresis for parasite characterization. *Methods Mol Biol* **21**, 361–382.

Benzecri, J. P. (1982). *L'analyse des Données: 2. L'analyse des Correspondances*. Paris: Dunod.

Boekhout, T. & Bosboom, R. W. (1994). Karyotyping of *Malassezia* yeasts: taxonomic and epidemiological implications. *Syst Appl Microbiol* **17**, 146–153.

Boekhout, T., Kamp, M. & Guého, E. (1998). Molecular typing of *Malassezia* species with PFGE and RAPD. *Med Mycol* **36**, 365–372.

Bond, R. & Anthony, R. M. (1995). Characterization of markedly lipid-dependent *Malassezia pachydermatis* isolates from healthy dogs. *J Appl Bacteriol* **78**, 537–542.

Coustau, C., Renaud, F. & Delay, B. (1991). Genetic characterization of the hybridization between *Mytilus edulis* and *Mytilus galloprovincialis* on the Atlantic coast of France. *Mar Biol* **111**, 87–93.

Coutinho, S. D. A. & Rodrigues Paula, C. (1997). Biotyping of *Malassezia pachydermatis* strains using the killer system. *Rev Iberoam Micol* **15**, 85–87.

Cunningham, A. C., Leeming, J. P., Ingham, E. & Gowland, G. (1990). Differentiation of three serovars of *Malassezia furfur*. *J Appl Bacteriol* **68**, 439–446.

Guého, E., Midgley, G. & Guillot, J. (1996). The genus *Malassezia* with description of four new species. *Antonie Leeuwenhoek* **69**, 337–355.

Guillot, J. & Guého, E. (1995). The diversity of *Malassezia* yeasts confirmed by rRNA sequence and nuclear DNA comparisons. *Antonie Leeuwenhoek* **67**, 297–314.

Guillot, J., Guého, E. & Chermette, R. (1995). Confirmation of the nomenclatural status of *Malassezia pachydermatis*. *Antonie Leeuwenhoek* **67**, 173–176.

Guillot, J., Guého, E., Chévrier, G. & Chermette, R. (1997). Epidemiological analysis of *Malassezia pachydermatis* isolates by partial sequencing of the large subunit ribosomal RNA. *Res Vet Sci* **62**, 22–25.

Holzschu, D. L., Phaff, H. J., Tredick, J. & Hedgecock, D. (1983). *Pichia pseudocactophila*, a new species of yeast occurring in necrotic tissues of columnar cacti in the North American Sonoran desert. *Can J Microbiol* **29**, 1314–1322.

Huang, H. P., Little, C. J. L. & Fixter, L. M. (1993). Effects of fatty acids on the growth and composition of *Malassezia pachydermatis* and their relevance to canine otitis externa. *Res Vet Sci* **55**, 119–123.

Kiss, G., Radványi, S. & Szigeti, G. (1996). Characteristics of *Malassezia pachydermatis* strains isolated from canine otitis externa. *Mycoses* **39**, 313–321.

Lebart, L., Morineau, A. & Warwick, K. A. (1984). *Multivariate Descriptive Statistical Analysis*. New York: Wiley.

Lewicka, K., Mallié, M. & Bastide, J. M. (1995). Genetic variability in the *Saccharomyces* sensu stricto complex revealed by multilocus enzyme electrophoresis. *Int J Syst Bacteriol* **45**, 538–543.

Lewontin, R. C. (1974). *The Genetic Basis of Evolutionary Change*. New York: Columbia University Press.

Marcon, M. J. & Powell, D. A. (1992). Human infections due to *Malassezia* spp. *Clin Microbiol Rev* **5**, 101–119.

Midgley, G. (1989). The diversity of *Pityrosporum* (*Malassezia*) yeasts *in vivo* and *in vitro*. *Mycopathologia* **106**, 143–153.

Midgley, G. (1993). Morphological variation in *Malassezia* and its significance in pityriasis versicolor. In *Dimorphic Fungi in Biology and Medicine*, pp. 267–277. Edited by H. Vanden Bossche. New York: Plenum.

Mittag, H. (1994). Fine structural investigation of *Malassezia furfur*. I. Size and shape of the yeast cells and a consideration of their ploidy. *Mycoses* **37**, 393–399.

Mittag, H., Brühl, R. & Hassel, U. (1994). An approach of the fine structure of *Malassezia furfur*. Proceedings of the XIIth Congress of the International Society of Human and Animal Mycology, March 13–18, 1994, Adelaide, Australia, PO6.3.

Nazzaro Porro, M., Passi, S., Caprilli, F., Nazzaro, P. & Morpurgo, G. (1976). Growth requirements and lipid metabolism of *Pityrosporum orbiculare*. *J Invest Dermatol* **66**, 178–182.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.

Nevo, E., Beiles, A. & Ben Shlomo, R. (1980). The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. *Lect Notes Biomath* **53**, 13–213.

Oxford, G. S. & Rollingon, D. (1983). *Protein Polymorphism: Adaptative and Taxonomic Significance*. The Systematics Association Special Volume 24. London: Academic Press.

Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J. & Britton-Davidian, J. (1987). *Manuel Technique de Génétique par Électrophorèse des Protéines*. Paris: Lavoisier.

Pujol, C., Reynes, J., Renaud, F., Mallié, M. & Bastide, J. M. (1993). Analyse génétique des souches de *Candida albicans* par électrophorèse des isoenzymes. *J Mycol Méd* **3**, 14–19.

Redline, R. W., Redline, S. S., Boxerbaum, B. & Dahms, B. B. (1985). Systemic *Malassezia furfur* infections in patients receiving intralipid therapy. *Hum Pathol* **16**, 815–822.

Richardson, B. J., Baverstock, P. R. & Adams, M. (1986). *Allozyme Electrophoresis. A Handbook for Animal Systematics and Population Studies*. New York: Academic Press.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–442.

Scott, D. W., Miller, W. H. & Griffin, C. E. (1995). Malasseziosis. In *Muller & Kink's Small Animal Dermatology*, 5th edn, pp. 351–354. Philadelphia: W. B. Saunders.

Selander, R. K. & Whittam, T. S. (1983). Protein polymorphism and the genetic structure of populations. In *Evolution of Genes and Proteins*, pp. 89–114. Edited by M. Nei & R. K. Koehn. Sunderland, MA: Sinauer.

Shaw, C. R. & Prasad, R. (1970). Starch gel electrophoresis of enzymes – a compilation of recipes. *Biochem Genet* **4**, 297–320.

She, J. X., Autem, M., Kotulas, G., Pasteur, N. & Bonhomme, F. (1987). Multivariate analysis of genetic exchanges between *Solea aegyptiaca* and *Solea senegalensis* (Teleosts, Soleidae). *Biol J Linn Soc* **32**, 357–371.

Simmons, R. B & Guého, E. (1990). A new species of *Malassezia*. *Mycol Res* **94**, 1146–1149.

Slooff, W. C. (1970). *Pityrosporum* Sabouraud. In *The Yeasts*, *a Taxonomic Study*, 2nd edn, pp. 1167–1186. Edited by J. Lodder. Amsterdam: North-Holland.

Tibayrenc, M. (1998). Beyond strain typing and molecular epidemiology: integrated genetic epidemiology of infectious diseases. *Parasitol Today* **14**, 323–329.

Tibayrenc, M., Kjellberg, F., Arnaud, J., Oury, B., Brenière, S. F., Dardé, M.-L. & Ayala, F. J. (1991). Are eukaryotic micro-organisms clonal or sexual? A population genetics vantage. *Proc Natl Acad Sci USA* **88**, 5129–5133.

Welbel, S. F., McNeil, M. M., Pramanik, A., Silberman, R., Oberle, A. D., Midgley, G., Crow, S. & Jarvis, W. R. (1994). Nosocomial *Malassezia pachydermatis* bloodstream infections in a neonatal intensive care unit. *Pediatr Infect Dis J* **13**, 104–108.

Yarrow, D. & Ahearn, D. G. (1984). *Malassezia* Baillon. In *The Yeasts*, *a Taxonomic Study*, 3rd edn, pp. 882–885. Edited by N. Y. W. Kreger-van Rij. Amsterdam: Elsevier.