Epidemiological aspects and genotypic characterization of strains of *Microsporum audouinii* isolated in the context of a Belgian National survey on anthropophilic tinea

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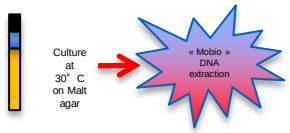
Objectives

- To investigate the epidemiological determinants responsible for the high number of anthropophilic dermatophytes received by the National Reference Centre for Mycosis of Liege (NRCL), during the last years. Indeed, the last two years, clinical cases of tinea capitis caused by *Microsporum audouinii (M. audouinii)*, have increased in Belgium.
- To perform a genotypic characterization by the Diversilab® system focusing on *M. audouinii.*
- To present results of the national survey launched between February 2013 and March 2014.

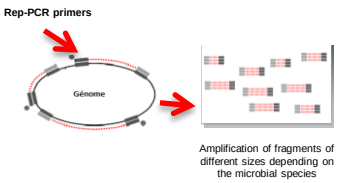
Methods

Population: A total of 117 strains of *M. audouinii* (116 clinical+ 1 reference strains) collected between March 2013 and February 2014 were included in the study. The strains were collected from different laboratories through Belgium but mainly were coming from Brussels. **Figure 1** describes the Diversilab® system (bioMérieux) used for genomic analysis of *M. audouinii* strains.

Step 1: Fungal culture + DNA extraction



Step 2: Rep-PCR



Step 3: Diversilab® analysis

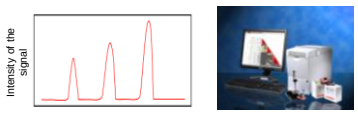


Figure 1: Description of the Diversilab® method.

Results

The 117 strains have been genotypically characterised by Diversilab® fingerprinting to visualize genomic variants between *M. audouinii* species. **Figure 2** represents the DNA fingerprints of these strains (Strains 1 to 26).

Figure 3 represents the scatterplot of all strains analysed showing the differenciation into 6 different groups. The genotypic analysis led thus to the distinction of several genotypic variants of *M. audouinii*. One of these variants was exclusively recovered from South Belgium (11 strains). The major group included 96 strains, well distributed in different Belgium locations.

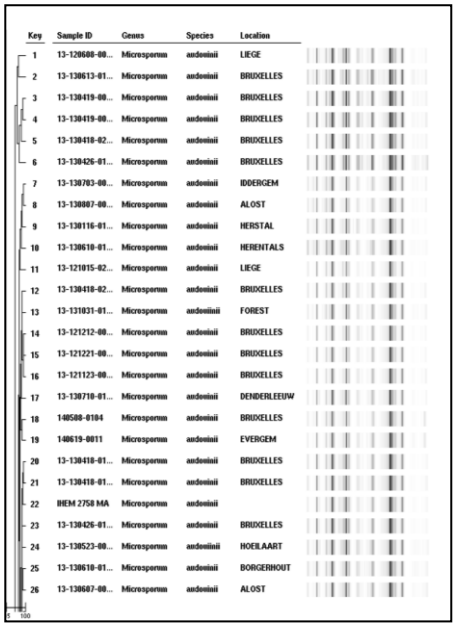


Figure 2: Dendrogram obtained after Diversilab® analysis of the strains N° 1 to 26.

Two other groups of three strains each were close to the major group but the analysis of the spectral superposition showed some differences between these groups (**See Figure 4**). The three last groups were clearly different from the major group but species identification was confirmed by ITS sequencing.

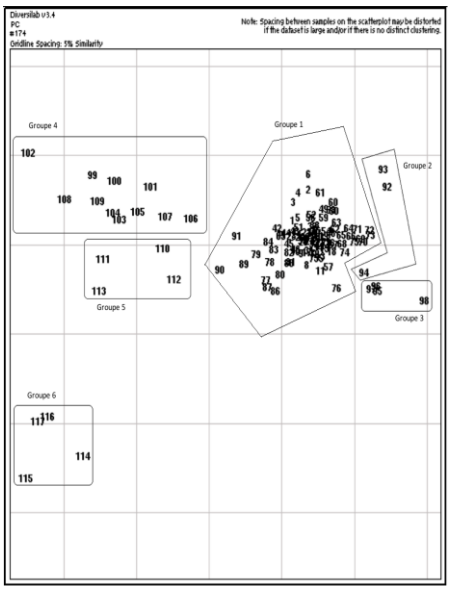


Figure 3: Scatter plots obtained after genomic analysis of strains by Diversilab® showing 6 different groups amongst *M. audouinii* strains.

Analysis of the epidemiological characteristics of the infected population (**see Table 1**) shows that the main age category concerns 5-9 year-old children (55,7%) with a sex-ratio M/F of 1.97. Data concerning the geographic origin of the family have been obtained in only 32,7% of the cases. It reveals that strains have been mainly isolated from patients with a Belgian nationality (44,7%) suggesting bias in the data collection. The geographic origin of the remaining group includes several African countries such as Congo (21,1%), Guinea (13,2%) and Cameroun (5,3%).

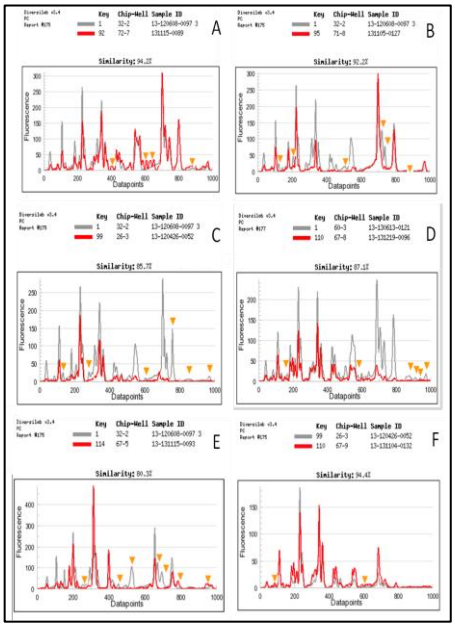


Figure 4: Rep-stacking of peaks obtained after Diversilab® analysis showing differences (yellow arrows) between strains of different defined groups.

		M. audouinii		
Variable	Geographical origin	N	Number (%)	p-value
Localisation		116		<0.0001
	Liège		29 (25.0)	
	Brussels		58 (50.0)	
	Dutch Brabant		5 (4.3)	
	Limbourg		2 (1.7)	
	Antwerpen		7 (6.0)	
	West Flanders		1 (0.9)	
	East Flanders		11 (9.5)	
	Hainaut		1 (0.9)	
	Walloon Brabant		2 (1.7)	
Age (years)		115		<0,0001
	0-4		29 (25.2)	
	5-9		64 (55.7)	
	10-14		17 (14.8)	
	>15		5 (4.3)	
Sex		116		0,0004
	Woman		39 (33.6)	
	Man		77 (66.4)	
Ethnical origin		38		<0,0001
	Belgium		17 (44.7)	
	Burundi		1 (2.6)	
	Congo		8 (21.1)	
	Guinea		5 (13.2)	
	Cameroun		2 (5.3)	
	Morocco		2 (5.3)	
	Mauritania		1 (2.6)	
	Rwanda		1 (2.6)	
	South Africa		1 (2.6)	
	Ethiopia		0 (0.0)	

Table 1: Epidemiological analysis of the datas obtained during the national survey.

Conclusions

The DiversiLab® system proved to be an efficient method to investigate the molecular epidemiology of dermatophytes infections, particularly the anthropophilic species increasing in Belgium. The present study shows that several groups of *M. audouinii* isolates co-exist in Belgium providing evidence of genetic heterogeneity inside this anthropophilic species. However, no clear correlation could be established between the appartenance to a group and epidemiological factors, such as the age or ethnical origin.