# Molecular characterisation of five metalloproteases (Mep1-5) and one subtilisin (Sub6) among *Microsporum audouinii* strains circulating in Belgium

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***Objectives***. Dermatophytes are producing proteases to digest tissue keratin and these enzymes have already been described as potential virulence factors in different zoonotic species such as *Trichophyton mentagrophytes* and *Microsporum canis*. In the present study, primers targeting five metalloproteases *Mep1-5 and* one subtilisin (*Sub6*) have been designed to screen a large scale of *Microsporum audouinii* strains isolated in Belgium in order to check the presence of these metalloproteases genes.

**Materials and methods**. Strains: 103 clinical strains collected in the National Reference Center, Liège (Belgium)/2 reference IHEM strains (BCCM, Brussels). Culture on Sabouraud dextrose agar: Identification by macroscopic and microscopic characteristics (+ITS sequencing if necessary) Culture on Sabouraud Dextrose Broth →DNA extraction by Maxwell 16 cell DNA purification kit preceded by enzymatic lysis using proteinase K for 20 minutes (Figure 1). Primers were newly designed based on nucleotide sequences of the genes *Mep1-5* available in GenBank database for the close related species *M. canis* and using primer Blast (NCBI-NIH). Sub6 primers were derived from an unpublished study about *M. canis* (Anne Mathy. B.Mignon et al, 2013, University of Liège). (Table 1).

**Results.** Among the 103 *M. audouinii* strains, the presence of at least one gene encoding for *Mep1-5* was revealed in 93% (96/103) with 80 % (87/103) being positive for the five *Meps* (1-5) (Figures 2-3). The detection was as followed: **89%** (92/103) for ***Mep1,* 90%** (91/103) for ***Mep2*** and ***Mep 5,* 85%** (88/103) for ***Mep3* & 92%** *(95/103) for* ***Mep4.*** In total, 7% (7/103) of the strains did not express any *Mep gene*. An internal control of amplification (ITS sequence) was also included to exclude a false negative result due to amplification inhibitors.

**Results (2)**

One Portuguese study *(A. Lemsaddek, Microbiol, 2010)* aiming the screening of metalloproteases genes in *M. audouinii* revealed also that *Mep4* was the most expressed one (100%). *Mep1* and *Mep5* were detected in 96%, *Mep3* in 91% and *Mep2* in 87% of the *M. audouinii* isolates. Concerning the screening of the *Sub6* gene, the analysis revealed that among the 103 *M. audouinii strains*, **87% (90/103)** of the isolates were positive for the *Sub6* gene. An internal control of amplification (ITS sequence) was also included to exclude a false negative result in the negative samples due to amplification inhibitors.

**Conclusion**

The presence of the *Mep1-5* genes in *M. audouinii* strains circulating in Belgium was confirmed in this study with 80% of the strains being positive for the five Meps. *Sub6* was also present in the main strains (87%). Fairly close percentages of expression of these genes let us think that all tested *Meps* and *Sub6* could be implicated in the virulence process of *M. audouinii* strains. The next step will be to confirm the *in vivo* expression of these metalloproteases in *M. audouinii.*

| | Primers | | Size (bp) |
|---|---|---|---|
| Target | Forward (5'-3') | Reverse (5'-3') | |
| Mep1 | AACTCTGCTACATG GCTAAG | CATAGTCATTACCGCC ATCT | 398 |
| Mep2 | CAGATGGTTCAATC CTTTGC | ATCCTTCTGGATGTAG ACGA | 514 |
| Mep3 | ACCTCTACTCCACT AACCTC | GTTGCATGGTTGACTA GAGA | 304 |
| Mep4 | CCTCTATTTTCCGT GGTTCA | AACATACATGAGAGG GTTCG | 801 |
| Mep5 | CCTACGTTGATGCT AAAAGC | TTACGGCCATGAGTGT ATTC | 730 |
| Sub6 | GGCCATTTTCTGAT GCTGGTATC | TTATTTGCCGTTGTA | 500 |
| ITS86/ ITS4 | GTGAATCATCGAAT CTTTGAA | TCCTCCGCTTATTGAT ATGC | 250-300 |

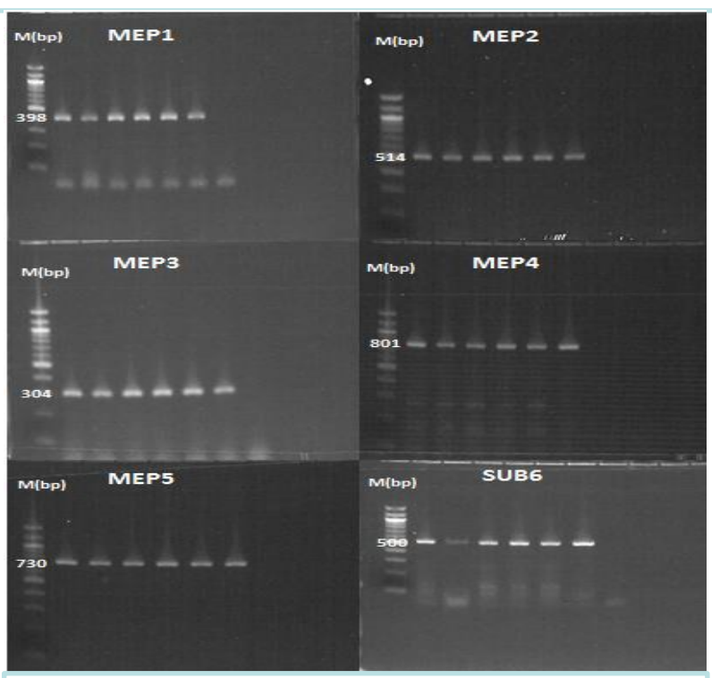


**Figure 1** : Electrophoresis gels with *Meps* and *Sub6*

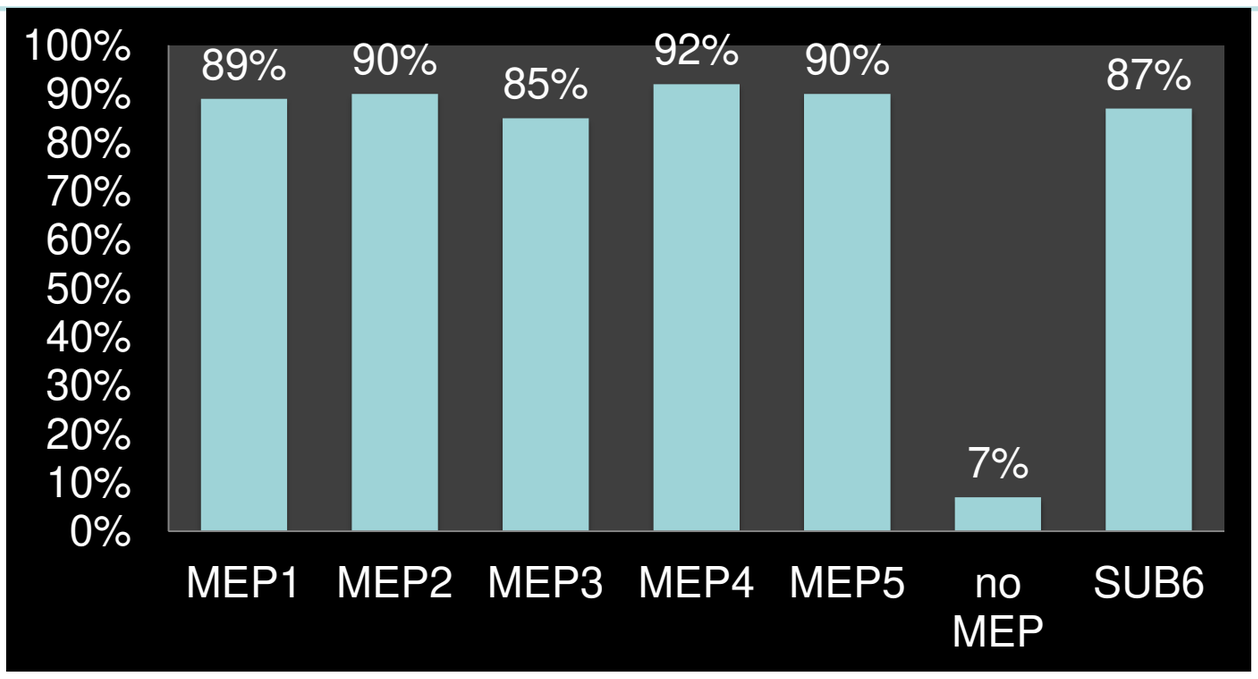


**Figure 2** : Occurrence of *Meps1-5 & Sub6* genes among the 103 *M. audounii* analysed strains

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