

Identifying indoor air *Penicillium* species: a challenge for allergic patients

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Abstract

Purpose. *Penicillium* is the most common mould isolated in housing. *Penicillium chrysogenum* is the only species tested by prick test or serology for allergic patients. The American Institute of Medicine has accepted *Penicillium* as an aetiological agent of rhinitis in children and adults and as an asthma agent in children. However, few studies have identified *Penicillium* in housing to the species level (354 species). Phenotypic identification is difficult. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) should be an alternative. The aim of this study was (1) to identify the *Penicillium* species present in dwellings in Eastern France and (2) to evaluate the reliability of MALDI-TOF MS for identification, by comparing it to DNA sequencing and phenotypic identification.

Methodology. Identification to the species level was performed by MALDI-TOF MS on 275 strains isolated from 48 dwellings. These results were compared to beta-tubulin gene sequencing and to the phenotypic aspects.

Results. Thanks to MALDI-TOF, 235/275 strains could be identified (85.5 %). Fourteen species were identified among 23 *Penicillium* species included in the Filamentous Fungi Library 1.0 (Bruker Daltonics). However, 72.2 % of the strains belonged to five main taxa: *P. chrysogenum* (27.3 %), *Penicillium glabrum* (22.9 %), *Penicillium commune* (11.3 %), *Penicillium brevicompactum* (6.5 %) and *Penicillium expansum* (4.2 %).

Conclusion. Complete coherence between MALDI-TOF MS and sequence-based identification was found for *P. chrysogenum*, *P. expansum*, *P. glabrum*, *Penicillium italicum* and *Penicillium corylophilum*. The main drawback was observed for *Penicillium crustosum*, which included 21 strains (7.6 %) that could not be identified using MALDI-TOF MS.

INTRODUCTION

Penicillium is one of the most common moulds isolated from housing. *Penicillium* is more common in indoor air than in outdoor air [1]. Increased relative humidity has been associated with higher concentrations of *Penicillium*, and visible mould as been associated with elevated levels of *Penicillium* [2]. *Penicillium* species release spores more easily than other species with dry spores, such as *Paecilomyces* or *Aspergillus versicolor* [3]. The genus *Penicillium* includes 354 species [4]. *Penicillium chrysogenum* (formerly *Penicillium notatum*) is the only one that is tested by prick test for allergic patients [5–7]. Penicillin initially produced by the species called *P. notatum* was recently identified as *Penicillium rubens*, which is similar to *P. chrysogenum*, and only distinguishable by DNA sequencing [8].

Some *Penicillium* species, such as *Penicillium roqueforti* and *Penicillium camemberti*, are used as ferments and contribute to the development of flavour in cheese [9]. By contrast, *Penicillium verrucosum* produces a very potent mycotoxin called ochratoxin A, which is considered to be harmful for human health if ingested [10, 11]. *Penicillium brevicompactum* from contaminated wallpaper can produce aerosolizable mycotoxin (i.e. mycophenolic acid) [12]. The Institute of Medicine (IOM) [13] considers *Penicillium* to be responsible for rhinitis in children and adults, and to be an asthma agent in children. Moreover, in some occupational circumstances, it can also contribute to the development of asthma in adults. *Penicillium variable* (Syn. *Talaromyces wortmanii*) has been associated with the development of asthma in 7-year-old children [14]

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Keywords: *Penicillium*; indoor air; species identification; MALDI-TOF; DNA sequencing; allergy.

Abbreviations: CYA, Czapek Yeast autolysate agar; DG 18, Dichloran Glycerol 18% medium; IOM, Institute of Medicine; ISHAM, International Society for Human and Animal Mycology; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight, mass spectrometry; MEA, 2% malt extract Agar; qPCR, quantitative real time Polymerase Chain Reaction.

and *Penicillium purpurogenum* (Syn. *Talaromyces purpurogenus*) was reported in stronger concentrations in the housing of children presenting respiratory wheezing [15]. *P. chrysogenum* conidia are major allergens for humans [16, 17]. Some species are agents of hypersensitivity pneumonitis (HP) (e.g. *Penicillium nalgiovense* for sausage workers, *P. brevicompactum* for wood workers and *Penicillium glabrum* for cork workers [18]. However, few studies have identified *Penicillium* in housing to the species level [19, 20]. Measuring the concentrations of fungal species present in the homes of allergic patients is considered to be an important part of allergy patient management [21]. *P. chrysogenum* has most often been reported as the main species isolated from dwellings [22]. However, the chrysogena section of *Penicillium* includes 18 species, including *P. chrysogenum* and *P. rubens* [23]. *P. rubens* has been described as the main contaminant species found in plasterboard in housing [24]. It is not feasible to identify the various species of the section *Chrysogena* for routine environmental monitoring of dwellings; consequently, in this study, *P. chrysogenum* was retained as broad identification for *Penicillium* belonging to the sect. *Chrysogena*.

Penicillium species are well described, but remain difficult to identify using microscopic characteristics [4, 25], unlike *Aspergillus* species, for example. Identification requires a number of different media that need to be checked for several weeks until some morphological characters appear [25]. Numerous morphological characters (the presence of exudates or diffusible pigments) vary from one strain to another within the same species. Molecular techniques (sequencing of DNA, qPCR) are expensive and difficult to use in routine practice.

On the other hand, using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) makes it possible to identify many moulds quickly [26]. The identification of 12 species of *Penicillium* using MALDI-TOF MS was achieved without any errors [27]. Currently, four commercial MALDI-TOF MS systems are available for routine mould identification. MALDI-TOF MS technology is less expensive, faster and easier to use than the current DNA-based identification gold standard. The most widely used system is from Bruker Daltonics (Bremen, Germany) [28, 29]. Some difficulties in identifying moulds can be attributed to the effects of culture conditions. In order to reduce these effects, a liquid-based cultivation method has been developed that standardizes the physiological status. Using the liquid cultivation method prevents the germination process and the formation of spores, which permits the creation of reproducible library entries. To obtain rapid identification, some medical laboratories use a direct deposit of part of the primary culture on four spots on the polished steel target plate, without cultivation on broth [29]. In the scope on housing mould identification for the management of allergic patients we do not have difficult time constraints. Hence we follow the manufacturer recommendations scrupulously, which is easy to do.

The aim of the present study was (1) to identify and assess the number of *Penicillium* species that are detectable in the indoor air of dwellings in Besançon (eastern France) and (2) to evaluate the reliability of MALDI-TOF MS for routine identification of *Penicillium* species, by comparing it to sequence-based (sequencing of the beta-tubulin DNA gene) and macro- and microscopic identification methods.

The risk of aggregating all species of *Penicillium* into the species *P. chrysogenum* (the only species tested by allergists) will be discussed.

METHODS

Nomenclature

In order to inform allergists about the *Penicillium* subsp. present in their patients' homes, we have chosen, in accordance with the recommendations of ISHAM [30], to use the name *Penicillium* rather than the name of the teleomorph (i.e. genus *Talaromyces*) that is used for certain species but is less common among allergists. By means of MALDI-TOF, we are striving to progress beyond the species *Penicillium chrysogenum* to include a larger number of species that can be linked to the symptoms of allergic diseases. This pragmatic approach does not call into question the molecular classification.

Origin of strains

To assess the influence of composting, air and dust samples from 38 dwellings in which household waste composting is performed and from 10 controls (a total of 26 apartments and 22 individual houses) were analysed by culture on dichloran glycerol 18 % medium (DG18) (Oxoid, Basingstoke, Hampshire, UK) and incubated for 1 week at 30 °C [31]. The air was either sampled by an MAS 100 impactor (Merck, Darmstadt, Germany) on DG18 medium or collected by a CIP 10 personal rotating cup sampler (Arelco, Fontenay sous Bois, France). The dust was sampled by an electrostatic dust collector in each dwelling [31, 32]. The *Penicillium* strains ($n=275$) obtained from air or dust samples were stored at -80 °C on 2 ml brain heart infusion (BHI) (BBL, Becton Dickinson, Sparks, MD, USA) medium with 50 % glycerol (v/v).

Identification by MALDI-TOF MS

The strains of *Penicillium* were isolated on DG18 and selected on macroscopic and microscopic aspects. The strains were then cultivated for 48 h in agitated liquid BHI medium on a wheel at room temperature (RT). The fungi were collected by centrifugation at 13 000 r.p.m. The pellet was washed three times with 1 ml of distilled water, and then transferred to a 1.5 ml Eppendorf tube containing 300 µl distilled water and 900 µl ethanol. The suspension was centrifuged at 13 000 r.p.m. for 3 min, and the pellet was dried at RT for 10–30 min. The pellet was then resuspended in 20 µl of 70 % formic acid. After a 10 min incubation at RT, an equal volume of acetonitrile was added. Samples were incubated again at RT for 10 min and subsequently centrifuged at 13 000 r.p.m. for 2 min. Supernatant (1 µl) was applied onto a

Table 1. Comparisons between MALDI-TOF MS and sequence-based identifications

		Beta-tubulin sequencing identification														
Number of sequenced strains		<i>P. chrysogenum</i>	<i>P. expansum</i>	<i>P. glabrum</i>	<i>P. italicum</i>	<i>P. corylophilum</i>	<i>P. brevicompactum</i>	<i>P. olsonii</i>	<i>P. roqueforti</i>	<i>P. citrinum</i>	<i>P. lanosum</i>	<i>P. commune</i>	<i>P. dierckxii</i>	<i>P. rugulosum</i>	<i>P. digitatum</i>	
Maldi-tof MS identification	<i>P. chrysogenum</i>	14														
	<i>P. expansum</i>	6	6													
	<i>P. glabrum</i>	6		6												
	<i>P. italicum</i>	1			1											
	<i>P. corylophilum</i>	2				2										
	<i>P. brevicompactum</i>	5					3	2								
	<i>P. olsonii</i>	1					1	0								
	<i>P. roqueforti</i>	4							3							
	<i>P. citrinum</i>	4								3						
	<i>P. lanosum</i>	4									0					
<i>P. commune</i>	7										0					
<i>P. dierckxii</i>	1											0				
<i>P. rugulosum</i>	1												0			
<i>P. digitatum</i>	3													0		
unidentified	33							1							0	
</																

Table 1. Continued

Beta-tubulin sequencing identification																
Maldi-tof MS identification	Number of sequenced strains	Beta-tubulin sequencing identification														
		<i>P. polonicum</i>	<i>P. nalgiovense</i>	<i>P. steckii</i>	<i>P. flavigenum</i>	<i>P. expansum</i>	<i>P. copitcola</i>	<i>P. citreonigrum</i>	<i>P. paxilli</i>	<i>P. purpurogenum</i>	<i>P. raistrickii</i>	<i>P. thymicola / P. hirsutum</i>	<i>P. corylophilum / P. steckii</i>	<i>P. adametzioides / P. scerotigenum</i>	<i>P. coralligerum</i>	unworkable sequences
<i>P. chrysogenum</i>	14															
<i>P. expansum</i>	6															
<i>P. glabrum</i>	6															
<i>P. italicum</i>	1															
<i>P. corylophilum</i>	2															
<i>P. brevicompactum</i>	5															
<i>P. olsonii</i>	1															
<i>P. roqueforti</i>	4															
<i>P. citrinum</i>	4			1 *												
<i>P. lanosum</i>	4		3		1 *											
<i>P. commune</i>	7	4														
<i>P. dierckxii</i>	1															1
<i>P. rugulosum</i>	1															1
<i>P. digitatum</i>	3															
unidentified	33				2	2	2	2	2	1	1	1	1	1	1	

Dark grey cells are expected results. Light grey cells correspond to exact concordance between MALDI-TOF MS and sequencing identifications. Asterisks correspond to MALDI-TOF MS identification with a score between 1.7 and 2.

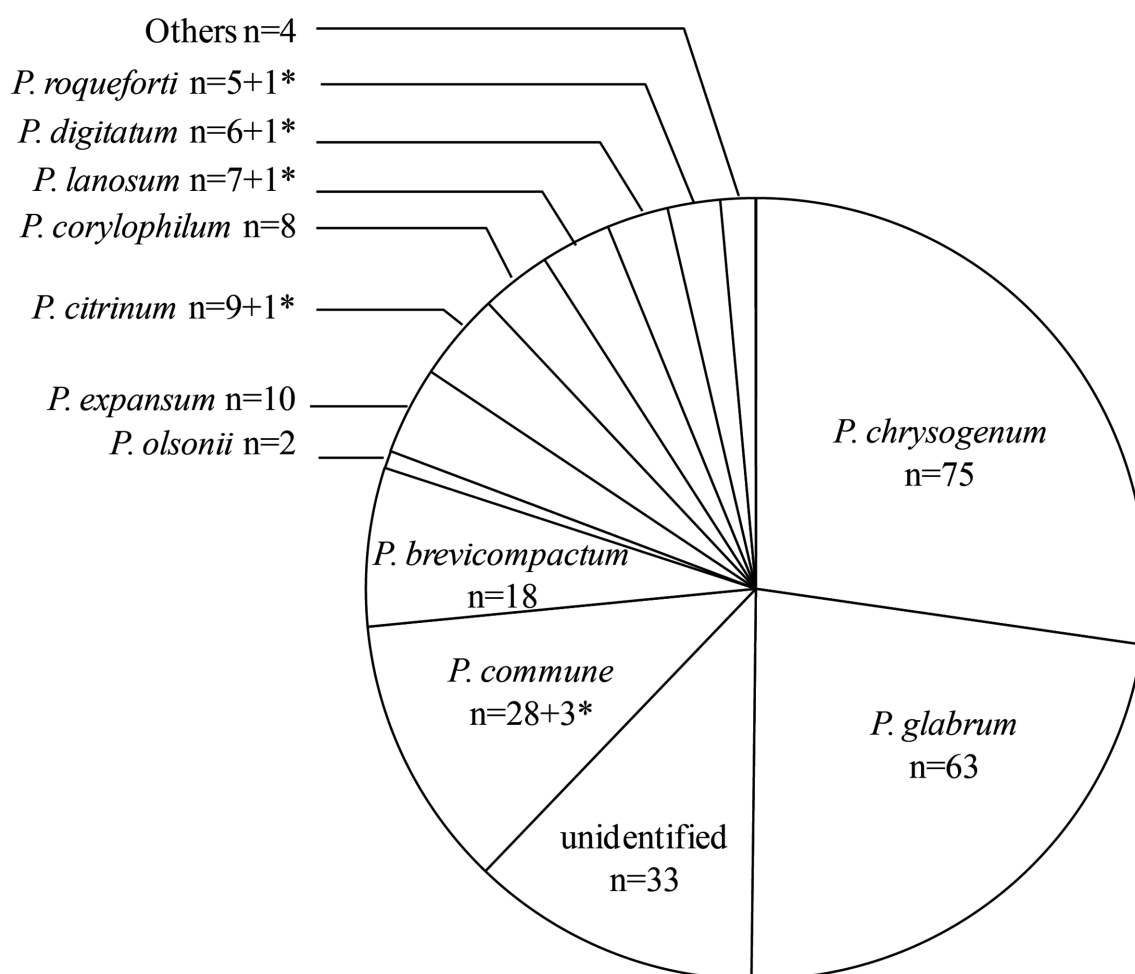


Fig. 1. MALDI-TOF MS identifications for the 275 analysed strains. *, correspond to MALDI-TOF MS identification with a score between 1.7 and 2. 'Others' are *Penicillium italicum* (n=1), *Penicillium dierckxii* (n=1) and *Penicillium rugulosum* (n=2).

polished steel target plate (Bruker Daltonics) and allowed to dry at RT before being overlaid with 1 µl of matrix solution (Bruker Daltonics). The acquisition and analysis of mass spectra were performed with a MALDI-TOF Microflex LT system (Bruker Daltonics).

The Bruker Bacterial Test Standard (Bruker Daltonics) was used for calibration in each series according to the manufacturer's instructions. Identification was obtained using the Bruker Daltonics Biotyper MALDI-TOF MS software package (version 3.1) with the Filamentous Fungi Library 1.2 (Bruker Daltonics), which includes 23 species of *Penicillium*. The interpretation was performed according to the manufacturer's recommendations (identification of the species was validated if the score was >2.0).

Identification by beta-tubulin gene sequencing

The beta-tubulin gene was sequenced for all the strains that could not be identified using MALDI-TOF MS and for those identified with a score between 1.7 and 2.0. Sequencing was also performed for some of the strains that were well identified using MALDI-TOF MS: 14/75 *P. chrysogenum*,

6/63 *P. glabrum*, 6/10 *P. expansum*, 5/18 *P. brevicompactum*, 4/28 *P. commune* and 1 to 3 strains for other less frequently observed species (Table 1). When possible, strains from the same species with different macroscopic aspects were selected for sequencing.

DNA was extracted from strains using the DNeasy Plant Mini kit (Qiagen, Milan, Italy). Molecular identification was performed by amplifying the beta-tubulin gene [33]. PCR products were purified with NucleoSpin gel and the PCR Clean-up kit (Macherey Nagel, Duren, Germany). Sequences were determined by an Applied Biosystems 3130 Genetic Analyzer (Thermo Fisher, Waltham, MA, USA) and compared to reference sequences in the GenBank database.

Phenotypic identification

Phenotypic identification was carried out by observing the grown colonies from the isolation medium (DG18), but also by observing the sub-culture on Czapek yeast autolysate agar (CYA) (Oxoid, Basingstoke, Hampshire, UK) and on 2 % malt extract agar (MEA) (Oxoid) after incubation at 25 °C. This

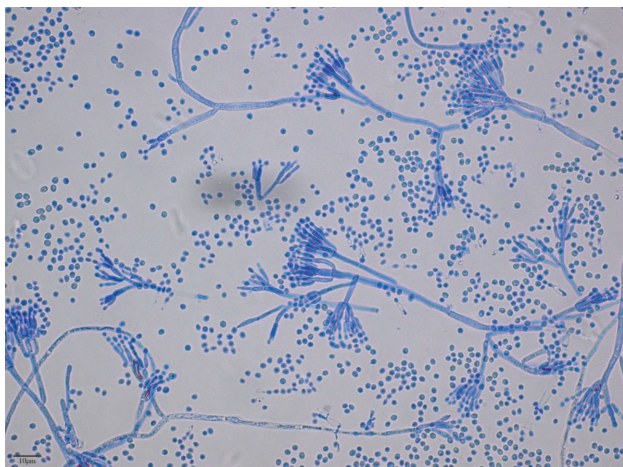


Fig. 2. Microscopic aspects of *Penicillium chrysogenum* (objective $\times 40$ and ocular $\times 12.5$). Scale indicated by a black drawing (10 μm).

incubation period lasted 3 weeks in order to allow the exudate and diffusible pigments to develop [25, 34]. An 'agar water' medium (2 % agar in water) (Difco Becton Dickinson, Sparks, MD, USA) was added to develop one-layer cultures on a slide. The objective was to check if MALDI-TOF MS and sequence-based identifications were coherent with the macroscopic and microscopic aspects observed from grown colonies.

RESULTS

Identification by MALDI-TOF MS

An identification was found for 242/275 strains, among which 235 (85.5 %) were identified with a score >2.0 and 7 (2.5 %) with a score between 1.7 and 2.0. Thirty-three strains (12 %) could not be identified by MALDI-TOF MS.

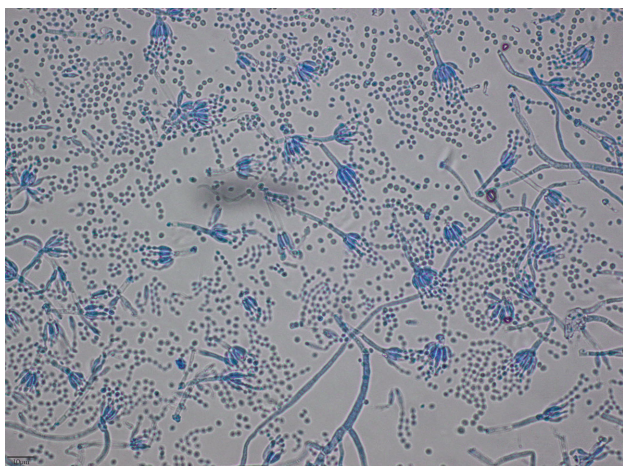


Fig. 3. Microscopic aspects of *Penicillium glabrum* (objective $\times 40$ and ocular $\times 12.5$). Scale indicated by a black drawing (10 μm).

Fourteen different species, among the 23 *Penicillium* species names in the database, were identified in our set of strains; 72.2 % of them belonged to five main taxa: *P. chrysogenum* (27.3 %), *P. glabrum* (22.9 %), *Penicillium commune*, (11.3 %), *P. brevicompactum* (6.5 %) and *Penicillium expansum* (4.2 %) (Fig. 1).

Identification by beta-tubulin gene sequencing

Ninety-two strains were sequenced for the beta-tubulin gene: 52 strains correctly identified by MALDI-TOF MS (score >2.0), 7 strains misidentified with a low score (between >1.7 and <2.0) and 33 strains not identified by MALDI-TOF MS (Table 1). Two out of the 92 sequences were not exploitable. The sequencing allowed the identification of 29 species/complex species among the 90 interpretable sequences (Table 1). Every sequence-based identification was checked using macroscopic and microscopic characters. Molecular identification was coherent with phenotypic identification for all the strains.

Comparisons between MALDI-TOF MS and sequence-based identification

Fifty-two of the 90 sequenced strains were either not identified or misidentified by MALDI-TOF MS.

Concordance between MALDI-TOF MS and sequencing identification

Complete concordance between MALDI-TOF MS (with a score >2.0) and sequence-based identification was observed for: *P. chrysogenum*, *P. expansum*, *P. glabrum*, *P. italicum* and *Penicillium corylophilum* (29 analysed strains, in light grey in (Table 1).

Five strains identified as *P. brevicompactum* by MALDI-TOF MS with a score >2 were placed in either the taxon *P. brevicompactum* ($n=3$) or in *Penicillium olsonii* ($n=2$) when using the sequencing method. One strain identified with MALDI-TOF as belonging to the *P. olsonii* species was identified as *P. brevicompactum* by sequencing.

Partial concordance was also found for *P. roqueforti* and *Penicillium citrinum*. For three strains of each of these two species, the sequencing and MALDI-TOF MS identification (scores >2.0) were concordant. For two other strains, the results were discordant: the first one was identified as *P. roqueforti* by MALDI-TOF MS with a score <2.0 , while it was identified as *P. crustosum* by sequencing. The second was identified as *P. citrinum* by MALDI-TOF MS with a score <2.0 whereas it was identified as *Penicillium steckii* by sequencing (Table 1).

Discordance between MALDI-TOF MS and sequencing identification

Discordance between MALDI-TOF MS and sequencing was found for *Penicillium lanosum* and *P. commune*, which were identified as *Penicillium nalgiovense* and *Penicillium polonicum*, respectively, by sequencing, although the score was >2 for all the strains sequenced ($n=3$ for

P. lanosum; $n=4$ for *P. commune*). Three strains identified as *Penicillium digitatum* by MALDI-TOF MS were identified as *P. crustosum* by sequencing.

No MALDI-TOF MS identification

Among the 33 strains that could not be identified by MALDI-TOF MS, 14 were identified as *P. crustosum* by sequencing. Among the 19 other strains, there were 16 strains from 11 different species not yet registered in the Bruker Daltonics Filamentous Fungi Library (Table 1). The three others comprised one strain of *P. olsonii* and two strains of *P. glabrum*.

MALDI-TOF MS never identified *P. crustosum*, although it is in the database, and our series included 21 *P. crustosum* strains according to sequence-based identification. To improve identification of the two common species that are difficult to identify using MALDI-TOF MS, new mass spectra for *P. crustosum* ($n=4$) and *P. polonicum* ($n=4$) were added to the database in our laboratory. Species identifications were confirmed by sequencing of the beta-tubulin gene for all eight strains before the mass spectra were included in the database.

DISCUSSION

The MALDI-TOF system together with the Bruker Daltonics database make it possible to identify 23 different species of *Penicillium*. The biodiversity of the *Penicillium* species is

variable according to dwellings. Twenty-nine different species of *Penicillium* were identified in the housing ($n=48$) in the town of Besançon (eastern France).

In our study, 72.2 % of the strains identified by MALDI-TOF MS belonged to 5 of these 23 species, but 14.5 % ($n=40/275$) of the strains could not be identified because of no response ($n=33$) or insufficient score ($n=7$). Currently, the main difficulty is to correctly identify *Penicillium* by MALDI-TOF MS to the species level because although *P. crustosum* represents the most common species, it could not be identified by MALDI-TOF MS in our series. In other words, it is underestimated by MALDI-TOF, which does not recognize it using the recorded profiles. Indeed, 14 strains could not be identified and 7 strains were misidentified (4 strains as *P. commune* or *P. roqueforti*, with a score <2.0 , and 3 strains as *Penicillium digitatum*, once with a score >2.0 and once with one <2.0). Moreover, *P. polonicum* was never identified using this technique, because of a systematic misidentification with *P. commune*. Since we found, using the sequence-based method, that these two species represented respectively 21/52 (40.4 %) and 4/52 (7.7 %) of the strains not identified or misidentified by MALDI-TOF MS, we added MS profiles of *P. crustosum* and *P. polonicum* strains isolated from housing recruited in our study to our in-house databank. This will be helpful in identifying the most common *Penicillium* species in our routine practice of mycological surveys in housing more quickly (Figs 2 and 3).

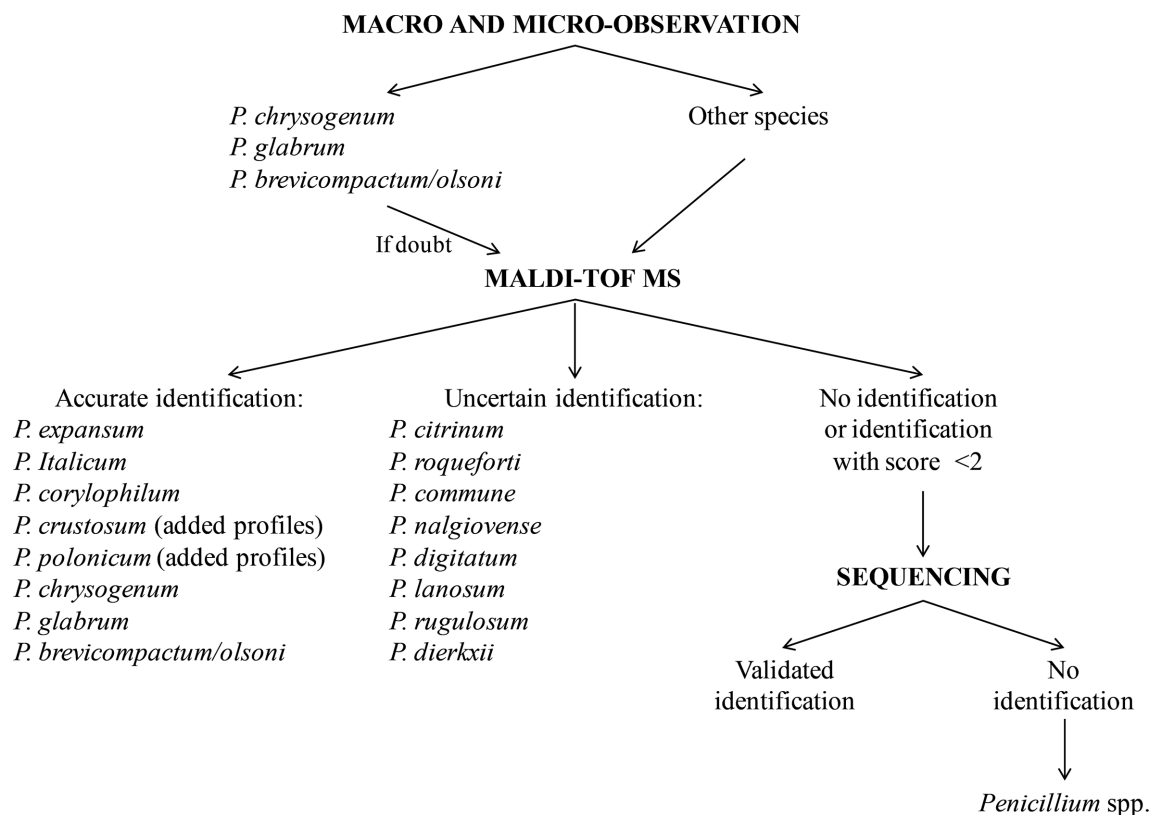


Fig. 4. Strategy for *Penicillium* identification to the species level for indoor strains.

Table 2. *Penicillium* species (listed in this series) known to be allergenic

Species	Pubmed*	Study subjects
<i>P. chrysogenum</i>	57	Antigen studies (Peni ch13, ch18), IgE cross-reactions, IgG, asthma risk and protection against asthma, sinusitis, sick building syndrome, dwelling, archives, domestic hypersensitivity pneumonitis (HP), HP (sausage)
<i>P. citrinum</i>	22	Antigen studies, HP (potatoes, sawmills), Asian dwellings
<i>P. brevicompactum</i>	10	Asthma, dwellings, antigen studies (Pen b26), HP (mushroom)
<i>P. expansum</i>	8	Antigen studies
<i>P. commune</i>	5	HP (cheese)
<i>P. glabrum</i>	3	HP (cork)
<i>P. nalgioense</i>	2	HP (sausage, cheese)
<i>P. polonicum</i>	2	HP (museum)
<i>P. roqueforti</i>	2	HP (cheese)
<i>P. citreonigrum</i>	1	HP (peat)

Isolated in this series but without reference to allergies:

P. adametzioides, *P. copticola*, *P. coralligenum*, *P. corylophilum*, *P. crustosum*, *P. digitatum*, *P. dierckxii*, *P. hisutum*, *P. italicum*, *P. flavigenum*, *P. lanosum*, *P. olsonii*, *P. paxillii*, *P. purpurogenum*, *P. raistrickii*, *P. rugulosum*, *P. steckii* and *P. thymicola*.

Publications * PubMed research with key words: « name of species+allergies » 15.05.2018.

Another problem encountered was the identification of *P. olsonii*/*P. brevicompactum*. *P. olsonii* is a very similar species to *P. brevicompactum*, which is difficult to identify by microscopic characters. One of the morphological differences for *P. olsonii* is the presence of slightly wider metulae than those of *P. brevicompactum* [34]. It is likely that *P. olsonii* and *P. brevicompactum* were subject to cross-identification when the mass spectra were entered into the fungal library. Cross-identification may also explain designation errors in GenBank [35]. In housing surveys, identification as a group *P. brevicompactum*/*P. olsonii* could be acceptable until possible antigenic differences are revealed and justify distinguishing between these two species.

To optimize the identification of *Penicillium* species in indoor environments, an identification strategy was proposed (Fig. 4), as below.

- Identification of three species (*P. chrysogenum*, *P. glabrum* and the group *P. brevicompactum*/*P. olsonii*) based on macroscopic and microscopic aspects from the DG18 medium [57.5 % of the strains in our series ($n=158$)] to reduce costs and delays.
- Identification using MALDI-TOF MS with a confidence level requiring no further control of *P. expansum*, *P. italicum* and *P. corylophilum* (6.9 % of the strains)

using the Bruker Daltonics database, and of two additional species *P. crustosum* (7.6 %) and *P. polonicum* (1.5 %) using the new profiles generated during this study. In addition, *P. chrysogenum*, *P. glabrum* and the group *P. brevicompactum*/*P. olsonii*, can be identified systematically and accurately by MALDI-TOF MS. So, approximately three-quarters of the strains (73.5%) can be identified to the species or group level using morphological and MALDI-TOF MS methods (divided into nine species).

- For the remaining 26.5 % strains with uncertain or failed identification by MALDI-TOF MS, recourse could be made to an additional microscopic examination when the characteristics are very discriminatory [i.e. the case of *P. citrinum* (1.5 %)]. If not, beta-tubulin gene sequencing could be performed. The MALDI-TOF MS profile could be saved in the databank and validated as a new profile with several strains.

The strains that could not be correctly identified by MALDI-TOF MS belong to multiple independent species (almost as many taxa as strains). These species reflect environmental peculiarities and can thus be included in a separate group called 'other species' (*Penicillium* subsp.).

P. chrysogenum only represents 27.3 % of the strains in our series, while it is generally the only *Penicillium* tested for allergies by prick test or serology. As a result, *P. chrysogenum* is the most commonly cited species in connection with allergies [13], with 46 % of publications mentioning *P. chrysogenum* – its distribution, antigens or implications for human health (PubMed consultation, 15 May 2018, key words: 'each *Penicillium* species name' and 'allergies') (Table 2). The other species have never been reported in relation to asthma, except *P. citrinum*, which has been particularly studied in Asia, and has been mentioned in 18 % of the publications on PubMed (Table 2), but which only represents 3.6 % of our isolates. Although there are common antigen cross-reactions between *P. chrysogenum*, *P. citrinum* and *P. brevicompactum*, the role of *Penicillium* species other than *P. chrysogenum* should be better specified. The research for common antigens (i.e proteases) should allow us to better understand the allergenic potential of each species. Our findings show that *P. crustosum* should be investigated as a potential aetiological agent of asthma in our region and compared with the antigenic potential of the other species with known enzymatic characterization.

The use of *P. chrysogenum* alone to determine whether or not a patient is sensitive to *Penicillium* is unscientific and overly confident in an antigenic community that should include all the different species of *Penicillium*. There is no evidence that the same *Penicillium* species is the aetiological agent of asthma, conjunctivitis or rhinitis. A synergistic or cumulative effect of certain antigens common to different species or even different genera could play a role in the development or the exacerbation of the allergic diseases.

The role of *Penicillium* in allergic diseases related to indoor air requires identification to the species level. About three-quarters of the strains are easily recognizable to the species

level: four with microscopic observation or with MALDI-TOF MS and five more with the MALDI-TOF. MALDI-TOF MS can be a relevant and accurate tool to carry out these inventories. This technique would be even more successful if new MALDI-TOF MS profiles of *Penicillium* strains isolated within the framework of allergy or indoor exposure could be validated and stored in a common database, and then made available to research teams working on this subject.

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Conceptualization: Reboux G, Millon L. Methodology: Rocchi S, Vacheyrou M, Millon L. Validation: Reboux G, Rocchi S, Vacheyrou M, Millon L. Formal analysis: Rocchi S. Investigation: Rocchi S, Vacheyrou M. Resources: Vacheyrou M. Writing-original draft preparation: Reboux G, Rocchi S. Writing, review and editing: Reboux G, Rocchi S, Vacheyrou M, Millon L. Visualisation: Rocchi S. Supervision: Reboux G, Millon L.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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