

Allergy / Early View

LETTER | [Open Access](#) | 

The emerging pathogen *Paecilomyces variotii* - a novel and important fungal allergen source

Sandra Pfeiffer, Marianne Raith, Mariona Pascal, Rosa Maria Munoz-Cano, Clara San Bartolome, Katharina Nöbauer, Santiago Quirce, Ebrahim Razzazi-Fazeli, Margarete Focke-Tejkl ... [See all authors](#) ▾

First published: 13 November 2021

<https://doi-org.bibliosan.clas.cineca.it/10.1111/all.15176>

Funding information:

This work was supported by the Austrian Research Promotion Agency [project 856337].

To the Editor,

Fungal allergy represents a worldwide public health burden.¹ Owing to their ability to colonize and germinate in the respiratory mucosa, fungi can have a far greater impact on the patients' immune system than other respiratory allergen sources.¹ However, the lack of knowledge about allergenic fungi is one of the factors that contribute to a general underdiagnosis of fungal allergy.² In the present study, we investigated the allergenic potential of *Paecilomyces variotii*, a heat-resistant mold ubiquitously found in soil, decomposing organic material, food products, and clinical samples, that is regarded as an emerging cause of serious, sometimes life-threatening, infections, and is often detected in the indoor environment.³⁻⁶ Even though *P. variotii* has already been suggested as an aeroallergen source and occupational hazard, no allergens have yet been identified or characterized by this species.⁷

IgE immunoblots, performed with four different pools of sera from mold-sensitized patients (Table [S1](#)), showed the presence of several IgE-reactive proteins in *P. variotii* extract (Figure [1A](#)), suggesting the species' high allergenic potential (no reaction was observed in immunoblots incubated with the detection antibody only—data not shown). Mass spectrometry analysis led to the identification of the first three *P. variotii* allergens, which interestingly all represent enzymes. Protein A (~36 kDa) was identified as a transaldolase, an enzyme that is already known as an allergen in different fungal species.⁸ Protein B (~37 kDa) was identified as a glyceraldehyde 3-phosphate dehydrogenase, which represents an important allergen in various sources,⁹ and Protein C (~48 kDa) was identified as an enolase.

Enolases have also already been identified as highly conserved and cross-reactive allergens in various allergen sources including different fungi.^{10, 11} The proteins were submitted to the WHO/IUIS Allergen Nomenclature Subcommittee and were tentatively assigned the allergen names Pae v 1^a, Pae v 2^b, and Pae v 6^c. The IgE-reactive protein bands marked D to H still have to be identified by peptide mass fingerprinting.

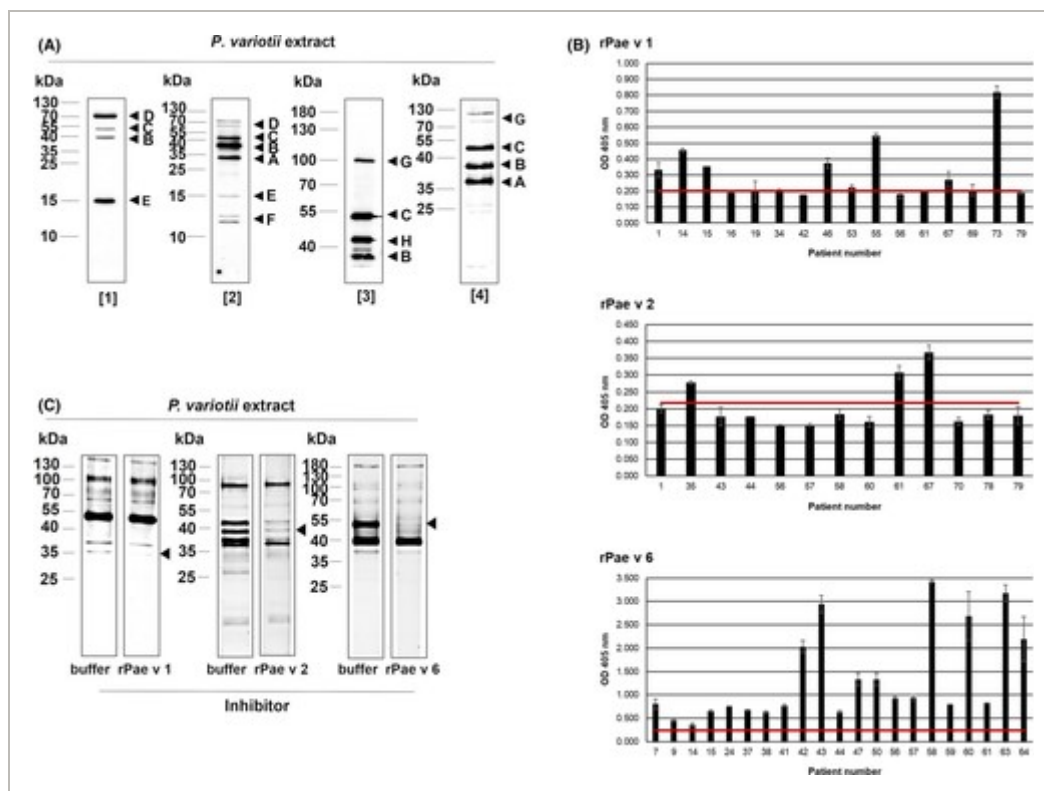


FIGURE 1

[Open in figure viewer](#) | [PowerPoint](#)

Identification and characterization of IgE-reactive proteins from *Paecilomyces variotii*. (A) Nitrocellulose-blotted proteins from *P. variotii* were exposed to four different pools of sera (pool 1: sera 1, 2, 26–28, and 65; pool 2: sera 3, 29–31, 40, 66, and 72; pool 3: sera 4–6, 48, 52, and 54; pool 4: sera 53, 56–61, 67, 77, and 78) from mold-allergic patients. IgE-reactive proteins identified by mass spectrometry are marked with A, B, and C; not yet identified bands are marked with D, E, F, G, and H. Molecular weight markers are indicated in the left margins. (B) Determination of IgE reactivity of rPae v 1, rPae v 2, and rPae v 6 by ELISA using sera from mold-sensitized patients. Results are displayed as mean OD values, and standard deviations from two technical replicates are indicated as error bars in the bar charts. The red line represents the assay's cutoff value, which was calculated from the mean OD values plus two times the standard deviation of sera from three non-allergic individuals. C, Inhibition immunoblots: blotted proteins in *P. variotii* extracts were exposed to different pools of sera (rPae v 1: sera 55 and 73; rPae v 2: sera 35, 39, and 61; and rPae v 6: sera 25, 57, 71, and 74–76), which had been pre-incubated with rPae v 1, rPae v 2, or rPae v 6, or, for control purposes, with buffer only. Molecular weight markers are indicated in the left margins. Arrows mark the molecular weights of the natural allergens Pae v 1, Pae v 2, and Pae v 6 in the fungal extract

Full-length cDNAs coding for the *P. variotii* proteins Pae v 1, Pae v 2, and Pae v 6 were

generated, cloned into a bacterial expression vector, and recombinant proteins were produced in *Escherichia coli* with a C-terminal hexa-histidine-tag. Reducing and non-reducing SDS-PAGEs (Figure [S1](#)) and immunoblots, performed with an anti-histidine-tag antibody, verified the purity of the recombinant proteins and showed that all proteins possess the capability to form disulfide bridges and to oligomerize under non-reducing conditions (Figure [S1](#)). Moreover, circular dichroism spectroscopy analysis of the recombinant allergens showed far-UV spectra with broad minima between 210 and 220 nm, which suggested the presence of both, alpha-helical structures, and beta-sheets, and indicated that the proteins represent properly folded molecules (Figure [S2](#)).

ELISAs performed with sera from mold-allergic patients showed the IgE-binding capacity of the three recombinant proteins. With OD values of more than 3.0 (Figure [1B](#)), the enolase rPae v 6 showed the highest levels of IgE reactivity, whereas the IgE reactivities of rPae v 1 (maximum OD value of 0.8) and rPae v 2 (maximum OD value of 0.390) were considerably lower. Furthermore, IgE inhibition immunoblots, where nitrocellulose-blotted protein extracts from *P. variotii* were exposed to serum pools that had been pre-incubated with either one of the recombinant proteins or, for control purposes, with buffer only, showed that the recombinant allergens rPae v 1, rPae v 2, and rPae v 6 were able to significantly reduce patients' IgE-binding to their natural counterparts (Figure [1C](#)). This indicates that the recombinant molecules contain the IgE-binding epitopes of their natural counterparts and suggests that rPae v 1, rPae v 2, and rPae v 6 could be used as tools for *in vitro* diagnosis of *P. variotii* sensitization. Pre-incubation with rPae v 2, a GAPDH, caused the reduction of IgE-binding to several proteins with molecular weights of 25–45 kDa. This suggests the presence of different Pae v 2 variants with different posttranslational modification and Pae v 2 degradation products in the natural fungal extract. The presence of GAPDH variants, which can also influence the molecule's different functions, has already been suggested in the literature.¹²

Analysis of the prevalence of IgE recognition in a cohort of patients, sensitized to different mold species, by ELISA revealed different frequencies of IgE reactivity. In Figure [S3](#), the presence (in green) or the absence (in red) of IgE-binding to the recombinant allergens is displayed for each patient. rPae v 1 was recognized by 22% (11 out of 49) of the tested patients, rPae v 2 by 6% (3 out of 50), and 68% of the tested patients (39 out of 57) reacted with rPae v 6. Among the patients tested with all three recombinant allergens, rPae v 1 was recognized by 22% (9 out of 41), rPae v 2 by 5% (3 out of 41), and rPae v 6 by 61% (25 out of 41) of the patients. Based on these results, rPae v 1 and rPae v 2 can be regarded as minor allergens of *P. variotii*, whereas rPae v 6 can be classified as a major mold allergen. Furthermore, evaluation of the allergenic activity of the recombinant major allergen rPae v 6 in basophil activation tests (BATs) showed a dose-dependent expression of CD63 in basophils from patient 4, suggesting the molecule's biological activity (Figure [S4](#) in the supporting information).

The availability of an anti-enolase antibody allowed us to further investigate the release

kinetics of the enolase Pae v 6 from *P. variotii* spores. The experiments showed that the spores from *P. variotii* immediately released this major allergen upon exposure to a humid milieu (Figure S5 in the supporting information). It can thus be expected that Pae v 6 would be rapidly secreted when the spores get in direct contact with the mucosa, leading to immediate allergic symptoms on the accessible mucosa surfaces such as the conjunctiva and the nose.

In conclusion, during this study the strong IgE reactivity of *Paecilomyces variotii* was demonstrated. Furthermore, the species first three allergens, tentatively designated Pae v 1, Pae v 2, and Pae v 6, were identified, recombinantly produced, and characterized regarding their biochemical and immunological features. It was shown that the three recombinant allergens represent IgE-reactive molecules, with Pae v 1 and Pae v 2 representing minor fungal allergens, whereas Pae v 6 can be classified as a major fungal allergen. We are aware that the use of sera from patients allergic to known fungal allergen sources is a limitation of our study, because, in this way, it was much more likely to identify highly conserved, potentially cross-reactive allergens rather than species-specific allergenic molecules. Indeed, one of the three identified allergens is the highly conserved fungal pan-allergen enolase. However, since *P. variotii* has not yet been thoroughly investigated as an allergen source, it was not possible to overcome this limitation. Nevertheless, the obtained data together with the facts that exposure to *P. variotii* represents an important component of the indoor mycobiota⁵ and is increasingly recognized as an important cause of infections¹³ suggest that this species should be considered as a potential allergen source and should be included in routine fungal allergy diagnosis.

The description of the used methods can be found in the supporting information.

ACKNOWLEDGEMENTS

The authors would like to thank Kay Holleis, MSc, from the Molecular Biotechnology Section, FH Campus Wien for his support during this study.

CONFLICT OF INTEREST

All authors declared no conflict of interest.

Supporting Information



Filename	Description
all15176-sup-0001-FigS1.jpg JPEG image, 438.6 KB	Fig S1
all15176-sup-0002-FigS2.jpg JPEG image, 303 KB	Fig S2

Filename	Description
all15176-sup-0003-FigS3.jpg JPEG image, 133.7 KB	Fig S3
all15176-sup-0004-FigS4.jpg JPEG image, 32.4 KB	Fig S4
all15176-sup-0005-FigS5.jpg JPEG image, 34.4 KB	Fig S5
all15176-sup-0006-TableS1.docx Word document, 15.2 KB	Table S1
all15176-sup-0007-TableS2.docx Word document, 13.5 KB	Table S2
all15176-sup-0008-MethodS1.docx Word document, 29 KB	Method S1

Please note: The publisher is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing content) should be directed to the corresponding author for the article.

REFERENCES



- 1 Bartemes K, Kita H. Innate and adaptive immune responses to fungi in the airway. *J Allergy Clin Immunol*. 2018; **142**(2): 353- 363. doi:[10.1016/j.jaci.2018.06.015](#)
[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)
- 2 Simon-Nobbe B, Denk U, Pöll V, Rid R, Breitenbach M. The spectrum of fungal allergy. *Int Arch Allergy Immunol*. 2008; **145**: 58- 86. doi:[10.1159/000107578](#)
[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)
- 3 Walsh TJ, Groll A, Hiemenz J, Fleming R, Roilides E, Anaissie E. Infections due to emerging and uncommon medically important fungal pathogens. *Clin Microbiol Infect*. 2004; **10**: 48- 66. doi: [10.1111/j.1470-9465.2004.00839.x](#)
[Wiley Online Library](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)
- 4 Shelton BG, Kirkland KH, Flanders KW, Morris GK. Profiles of airborne fungi in buildings and outdoor environments in the United States. *Appl Environ Microbiol*. 2002; **68**(4): 1743- 1753. doi: [10.1128/AEM.68.4.1743-1753.2002](#)
[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)
- 5 Lukaszuk CR, Krajewska-Kulak E, Kulak W. Effects of fungal air pollution on human health. *Prog Health Sci*. 2011; **1**(2): 156- 164.
[Google Scholar](#)
- 6 Kobińska N, Mularczyk M, Kosiorowska K, et al. New strains of filamentous fungi isolated from construction materials. *EJPAU*. 2019; **22**(1).
<http://www.ejpau.media.pl/volume22/issue1/art-02.html>

[Crossref](#) | [Google Scholar](#)

7 Houbraken J, Verweij PE, Rijs AJMM, Borman AM, Samson RA. Identification of *paecilomyces variotii* in clinical samples and settings. *J Clin Microbiol*. 2010; **48**(8): 2754- 2761. doi: [10.1128/JCM.00764-10](https://doi.org/10.1128/JCM.00764-10)

[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

8 Chou H, Wu K-G, Yeh C-C, et al. The transaldolase, a novel allergen of *fusarium proliferatum*, demonstrates IgE cross-reactivity with its human analogue. *PLoS One*. 2014; **9**(7):e103488. doi: [10.1371/journal.pone.0103488](https://doi.org/10.1371/journal.pone.0103488)

[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

9 Benndorf D, Müller A, Bock K, Manuwald O, Herbarth O, von Bergen M. Identification of spore allergens from the indoor mould *Aspergillus versicolor*. *Allergy*. 2008; **63**: 454- 460. doi: [10.1111/j.1398-9995.2007.01603.x](https://doi.org/10.1111/j.1398-9995.2007.01603.x)

[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

10 Simon-Nobbe B, Probst G, Kajava AV, et al. IgE-binding epitopes of enolases, a class of highly conserved fungal allergens. *J Allergy Clin Immunol*. 2000; **106**(5): 887- 895. doi: [10.1067/mai.2000.110799](https://doi.org/10.1067/mai.2000.110799)

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

11 Pfeiffer S, Sandler P, Raith M, et al. Identification of *Ulocladium chartarum* as an important indoor allergen source. *Allergy*. 2021; **76**(10): 3202- 3206. doi:[10.1111/all.14999](https://doi.org/10.1111/all.14999)

[Wiley Online Library](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

12 Zaffagnini M, Fermani S, Costa A, Lemaire S, Trost P. Plant cytoplasmic GAPDH: redox post-translational modifications and moonlighting properties. *Front Plant Sci*. 2013; **4**:450.

[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

13 Sprute R, Salmanton-Garcia J, Sal E, et al. Characterization and outcome of invasive infections due to *Paecilomyces variotii*: analysis of patients from the FungiScope registry and literature reports. *J Antimicrob Chemother*. 2021; **76**(3): 765- 774. doi:[10.1093/jac/dkaa481](https://doi.org/10.1093/jac/dkaa481)

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

[Download PDF](#)

About Wiley Online Library

Privacy Policy

Terms of Use

Cookies

Accessibility

Publishing Policies

Help & Support

Contact Us

Training and Support

DMCA & Reporting Piracy

Opportunities

Subscription Agents

Advertisers & Corporate Partners

Connect with Wiley

The Wiley Network

Wiley Press Room

Copyright © 1999-2021 John Wiley & Sons, Inc. All rights reserved