

CASE REPORT

Case Report. *Monascus purpureus*—a new fungus of allergologic relevance**Fallbericht. *Monascus purpureus*: Ein neuer, allergologisch bedeutsamer Pilz**

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Key words. *Monascus purpureus*, allergenicity, red fermented rice.**Schlüsselwörter.** *Monascus purpureus*, Allergenität, rot fermentierter Reis.

Summary. Anaphylactic reactions to food containing allergens in the consumption or preparation of food are well known. However, allergy in the preparation of sausages has rarely been described. In the present study a 26-year-old-butcher was investigated who had a severe anaphylactic reaction developing sneezing, rhinitis, conjunctivitis, generalised pruritus, followed by widespread urticaria, Quincke's oedema and dyspnoe after starting to prepare sausages containing red yield rice. Red yield rice is produced by the fungus *Monascus purpureus*. It was the first time that *Monascus purpureus* could be shown as allergic agent by means of prick-to-prick test, Cellular Antigen Stimulation Test (CAST) and different immunoblots.

Zusammenfassung. Anaphylaktische Reaktionen gegenüber Nahrungsmitteln und den darin enthaltenen Allergenen sind gut bekannt. Allergien, die bei der Bereitung von Würsten auftreten, sind jedoch sehr selten. In der vorliegenden Studie wurde ein 26jähriger Fleischer untersucht, der anaphylaktische Reaktionen wie Niesen, Rhinitis, Konjunktivitis, generalisierten Pruritus, gefolgt von einer ausgedehnten Urtikaria, Quincke-Ödem und Dyspnoen entwickelte,

nachdem er bei der Herstellung von Wurst Kontakt mit rot fermentiertem Reis hatte. Rot fermentierter Reis stellt ein Produkt aus poliertem, gewaschenem Reis und dem Pilz *Monascus purpureus* dar. Mit Hilfe des Pricktests, des Zellantigenstimulationstests (CAST) sowie verschiedener Immunoblot-Untersuchungen konnte erstmals *Monascus purpureus* als Ursache für die aufgetretene allergische Reaktion nachgewiesen werden.

Introduction

Anaphylactic reactions to food containing allergens in the consumption or preparation of food are well known [1]. A lot of fungi are not the only reason for cutaneous, subcutaneous and systemic mycosis but they can also generate allergic reactions.

In the present study it was possible to show the allergic effects of *Monascus purpureus*. The fungus *M. purpureus* was discovered in 1884 by van Tieghem and described by Went and was known as a contaminating fungus on cereals, starch and silage in Europe.

However, in Asia, *M. purpureus* is used to prepare red fermented rice and is also used as a natural food additive and preservative. Red fermented rice is a traditional material which has been used in China since the Tang Dynasty. It is more commonly known as 'ang-kak' or 'koji' in Japan.

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Washed and cooked non-glutinous rice was mixed with *M. purpureus*. After solid fermentation for three weeks, the rice was dried and crushed, resulting in a red powder. This ground powder is traditionally known for its health-promoting qualities. It is shown in animal and human studies that *M. purpureus* effectively lowers serum lipid levels due to the microbial metabolites it contains, such as monacolin K with 3-hydroxy-3-methylglutaryl coenzyme (a reductase inhibitor activity [2–4]). Additionally, observed bacteriostatic effects (*Bacillus*, *Streptococcus*, *Pseudomonas*) have led to the use of *M. purpureus* fermentate, at least partially as a substitute for nitrite in meat preservation.

We present a 26-year-old-butcher who had a severe anaphylactic reaction developing sneezing, rhinitis, conjunctivitis, generalised pruritus, followed by widespread urticaria, Quincke's oedema and dyspnoea, after he had started to prepare certain kinds of Thuringia sausages containing red yield rice. He required emergency treatment with intravenous corticosteroids, theophylline, antihistamines and inhaled β_2 -mimetics followed by a detailed allergic diagnosis.

Materials and methods

The fungus *M. purpureus* was received from the University of Bonn (Department of Food Technology). After 2–3 weeks dark culture at 28 °C in Sabouraud-Glucose Agar 0, 9% NaCl-solution was added and the suspension was centrifuged (Fig. 1).

The crushing was performed by means of the Bead Beater (Biospec Product, Bartlesville, USA). After dialysis of the mixture the concentration of proteins was determined according to the BCA method of Pierce.

The extract was used in SDS gel electrophoresis and Westernblot investigations. Additionally, a paper disc was prepared with *M. purpureus* extract to detect serum IgE antibodies to *M. purpureus* by radioallergosorbent test (RAST).

The detection of serum IgE antibodies was additionally performed by AlaSTAT-Immunoblots (DPC Biermann, Bad Nauheim, Germany) in which immunoblots with *Aspergillus fumigatus* and α -amylase were used as controls.

The achieved extract from *M. purpureus* was used in skin prick tests (patient + 10 healthy controls) as well as in Cellular Antigen Stimulation Tests (patient + 5 healthy controls).

The Cellular Antigen Stimulation Test (CAST) was performed using EDTA blood by means of

dextrane sedimentation. After 90 min incubation at room temperature the upper phase was centrifuged with $130 \times g$ 15 min at 4 °C. The supernatant was removed and the cell pellet was resolved in 2 ml incubation buffer containing 270 μ l interleukin-3. Afterwards, 200 μ l of cell suspension were transferred into Eppendorf-tubes and stimulated with 50 μ l incubation buffer and interleukin-3 (200 μ l IB + 3 μ l IL-3). In more modern test kits, the stimulation buffer already contains the interleukin-3. Two extra tubes with 200 μ l cell suspension were stimulated with 50 μ l of monoclonal anti-IgE-antibody (Le 27) or with the monoclonal antibody of the high-affinity-IgE-receptor. 50 μ l of each allergen solution (red-fermented rice or extract of *M. purpureus*) were added. The stimulation was performed 40 min at 37 °C. After centrifugation at the same conditions described above the supernatant was frozen at –20 °C. The enzyme immuno assay must be carried out no later than 4 weeks. The CAST-ELISA is based on the recognition of leukotrien C4 as well as its two metabolites (leukotrien D4 and leukotrien E4) by a single monoclonal antibody with the same sensitivity and specificity [5–9]. By means of the CAST-ELISA all sulfidoleukotrienes can be measured generated by cellular antigen stimulation. Sulfidoleukotriene concentrations >200 pg ml^{–1} are significant for allergic reactions.

Results

The patient was tested with several food preservatives, spices and food additives in skin tests. A prick-to-prick test performed with red yield rice (Food Line GmbH, Offenbach, Germany) was positive. Skin-prick tests, as well as specific IgE antibodies, were negative to rice. A subsequent prick-to-prick test performed on the patient with the extract of *M. purpureus* provided a similar, positive result, whereas 10 healthy controls remained negative.

Cellular Antigen Stimulation Test (CAST) was positive to red yield rice and to extract of *M. purpureus* (Fig. 2).

The measured concentrations of sulfidoleukotrienes >500 pg ml^{–1} confirm the allergic reaction. The reproducibility of the results was tested by intra- and inter-assay variation of the CAST over several days and with different concentrations of *M. purpureus* extract. The 5 healthy controls remained negative showing sLT-levels <200 pg ml^{–1} (Fig. 3).

The allergen specific signal at 25 kDa was found in Westernblots with paper discs coated



Figure 1. Culture of *Monascus purpureus*. The fungus produces a water-soluble red pigment. See Cover Illustration of *mycoses* **44**, issue 9–10 (2001).

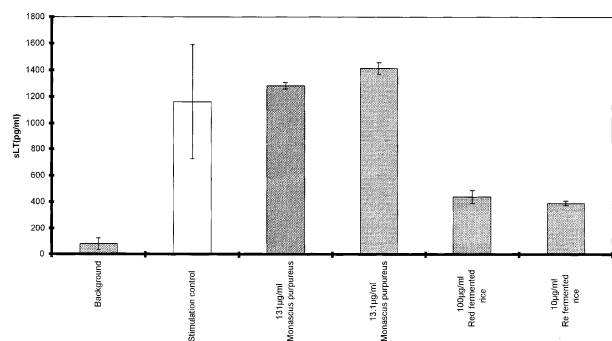


Figure 2. Cellular Antigen Stimulation Test (CAST) with extract of *Monascus purpureus* in the patient (CAST performed on several days).

with extract of *M. purpureus* (Dr Wahl, Allergo-pharma) as well as by means of AlaSTAT-Immunoblots (DPC Biermann, Bad Nauheim, Germany). The controls with *Aspergillus fumigatus* and α -amylase remained negative.

Conclusions

The reasons for the very strong anaphylactic reactions of our patient were determined using allergic diagnosis. This case highlights the importance of a detailed allergological assessment of natural allergens by *in vivo* and if available by *in vitro* tests.

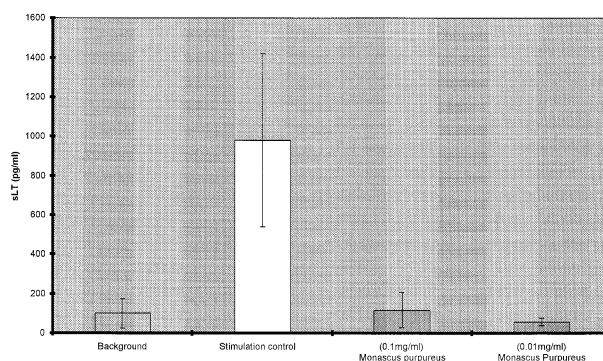


Figure 3. Cellular Antigen Stimulation Test (CAST) with extract of *Monascus purpureus* in 5 healthy control patients.

The corresponding results of skin tests, serum IgE-determination, CAST and immunoblots confirm the presence of an IgE-mediated allergy against *Monascus purpureus*. To our knowledge, this is the first documented case of anaphylaxis to red yeast rice due to an immediate type sensitivity to *M. purpureus* in which specific IgE antibodies could be shown.

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