

Analysis of the Allergen Expression of *Blomia tropicalis* and *Blomia kulagini* (Astigmata: Glycyphagidae) Cultures

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ABSTRACT Laboratory cultures of the mites *Blomia tropicalis* (van Bronswijk, Cock & Oshima) and *Blomia kulagini* (Zakhvatkin) were used to study the population dynamics of the mites and the kinetics of released allergens during the growth cycle. The analysis of extracts obtained after different incubation periods, by means of immunoblotting, and quantification of the major allergen Blo t 5, allowed definition of three different growth phases, demonstrating that mite cultures during the maximum growth (end of exponential growth curve–beginning maximum growth plateau) contain the largest amount of allergenic components as well as the highest Blo t 5 concentration.

KEY WORDS *Blomia tropicalis*, *Blomia kulagini*, mite allergens, mite growth, Blo t 5

Blomia tropicalis (van Bronswijk, Cock & Oshima) is the mite most prevalent in house dust samples in Colombia (Mulla et al. 1980, Puerta et al. 1991) and is a very common cause of allergy in cities at sea level (Puerta et al. 1993) and in cities up to 2,800-m altitude (Sánchez et al. 1990). In other tropical and subtropical areas, mites in the genus *Blomia* have been shown to be an important cause of sensitization (Jorge et al. 1980, Fernández-Caldas et al. 1990, García-Robaina et al. 1997, Yi et al. 1999). In the past few years, studies on the prevalence of mite sensitization and mite fauna in house dust samples from nontropical and subtropical regions have shown the increasing importance of *Blomia* as a cause of sensitization and allergy (Arias and del Hoyo 2002, Thomas et al. 2003) and suggest that this mite's distribution is not exclusively tropical (van Hage-Hamsten 1995). This is supported by the findings of Portús et al. (1976), Gómez et al. (1977), and Franjola and Rosinelli (1999), who reported the presence of *B. tropicalis* in northwestern Spain at sea level, *Blomia kulagini* (Zakhvatkin) in northwestern Spain at 1,200-m altitude in an alpine climate, and *Blomia tjiobdas* (Oudemans) in southern Chile.

Mite cultures are the most important source of allergenic material in the production of extracts used for the diagnosis and specific treatment of mite allergy, and the optimal culture conditions required to obtain the allergenic components should be exhaustively analyzed to guarantee the maximum diagnostic and therapeutic efficacy (Eraso et al. 1997).

The allergenic expression of *Dermatophagoides* cultures has been widely studied (Eraso et al. 1997), but there have been few reports on the growth of mites from the genus *Blomia* (Bronswijk et al. 1973, Yi et al. 1999), and no data could be found on *B. kulagini*.

The current study investigated the growth pattern of *B. tropicalis* and *B. kulagini*, the expression of the complete allergenic mosaic during the culturing period of both species, and also the kinetics of the individualized major allergen Blo t 5 during the growth curves.

Materials and Methods

Mite Culture. *B. tropicalis* (Central Science Laboratory, York, United Kingdom) and *B. kulagini* (Portús, Department of Parasitology, Faculty of Pharmacy, University of Barcelona, Spain) were cultured on a 1:1 (wt:wt) autoclaved mixture of commercial mouse meal (A.04, Panlab, Barcelona, Spain) and dried yeast powder. Thirty-six cultures per species (12 in triplicate) were prepared simultaneously by inoculating with 3,600 mites in 30 mg of medium. Cultures were incubated at 24–26°C and 75–80% RH, which was maintained by a saturated solution of sodium chloride (Eraso et al. 1997). Cultures were examined regularly (every 2 wk) to assess mite growth and to discard any cross-contamination. Manipulation of cultures was performed in a biological safety cabinet.

At 2-wk intervals, a small culture sample (50 mg) was used to determine the mite concentration (live and dead mites) by microscope count. Cultures were then processed to obtain the extracts.

Extract Preparation. Extraction from the culture medium and from the whole culture containing mites were performed by shaking for 6 h at 4–8°C in saline phosphate buffer, pH 7.2 (1 g of raw culture/10 ml of buffer). Extract suspension was clarified by centrifugation.

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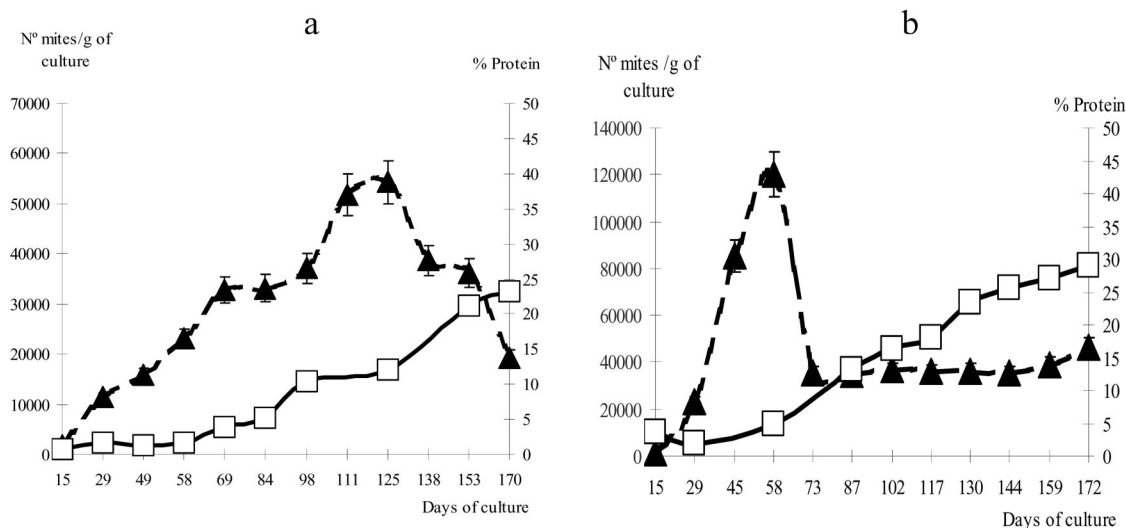


Fig. 1. Mite growth (▲) and protein content (□) of extracts in cultures of *B. tropicalis* (a) and *B. kulagini* (b). Mite growth (▲) indicates the total number of mites (living plus dead). Percentages of protein (□) were calculated subtracting the protein percentage of culture medium extract from the protein percentage of extracts obtained in each point (every 2 wk) of growth curve.

gation at $3,000 \times g$ for 30 min., and the supernatants filtered by cellulose AP20 filters (Millipore, Bedford, MA). The extracts were thoroughly dialyzed against distilled water at 4°C during 24 h, by using membranes with a 5,000 Da cut-off. Dialyzed material was centrifuged at $12,000 \times g$ for 30 min at 4°C and the supernatants were freeze-dried (Eraso et al. 1997).

Protein Determination. Protein concentrations were determined by the bicinchoninic acid method, according to Smith et al. (1985), by using bovine serum albumin as the standard.

Human Sera. Sera were obtained from 35 mite-sensitive patients from the coffee-producing region of Colombia, with histories of persistent allergic symptoms. The sera fulfilled the guidelines recommended by the European Academy of Allergy and Clinical Immunology for allergen standardization. All sera were screened to determine the specific IgE levels against *B. tropicalis* and *B. kulagini* extracts and showed high values (RAST class ≥ 2). To perform the immunological tests, a pooled serum sample was prepared using aliquots of each of the individual sera. Control sera were obtained from nonatopic individuals. Explicit consent was obtained from them.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) Immunoblotting. Protein separation was performed by SDS-PAGE by using polyacrylamide concentrations of 12.5 and 4% for separating and stacking gels, according to Laemmli (1970) as modified by Erasó et al. (1997).

Separated protein bands were electrophoretically transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore) by using a horizontal semi-dry transfer system (Towbin et al. 1979). Detection of immune complex was made according to Shen et al. (1988), with modifications during the developing

step by using chemoluminescent reagents (ECL+, Western blotting detection reagents, Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshire, England). Protein and allergen patterns were analyzed by means GEL COMPAR 4.0 (Applied Maths, Kortrijk, Belgium).

Major Allergen Determinations. Measurement of the major allergen Blo t 5 was performed by two-site monoclonal antibody (mAb) enzyme-linked immunosorbent assay, by using capture mAb 4G9, Biotine mAb 4D9, and recombinant Blo t 5 standard (IN-DOOR Biotech Ltd., Manchester, United Kingdom), according to Luczynska et al. (1989) as described by Erasó et al. (1997).

Results

The growth pattern of *B. tropicalis* and *B. kulagini* during the 24-wk period, and its correlation with the protein content of the extracts obtained every 2 wk are shown in Fig. 1a and b, respectively.

The growth pattern of both species could be divided in three distinct phases, based on the ratio $R_{l/d}$ (number of living mites/number of dead mites): a first latent phase with $R_{l/d}$ values >50 (days 1–20 for *B. tropicalis* and 1–30 for *B. kulagini*). A second exponential phase with $R_{l/d}$ values 50–10 (days 21–130 for *B. tropicalis* and 31–58 for *B. kulagini*). A third drop stage with $R_{l/d}$ values <10 (days 131–170 for *B. tropicalis* and 59–172 for *B. kulagini*). In no case could be detected living *B. tropicalis* after the day 170 and living *B. kulagini* after the day 100.

B. tropicalis showed very similar protein patterns in extracts from cultures obtained after 58 and 120 d of incubation (93% homology), revealing 12 to 13 different components (molecular weight range 100–14

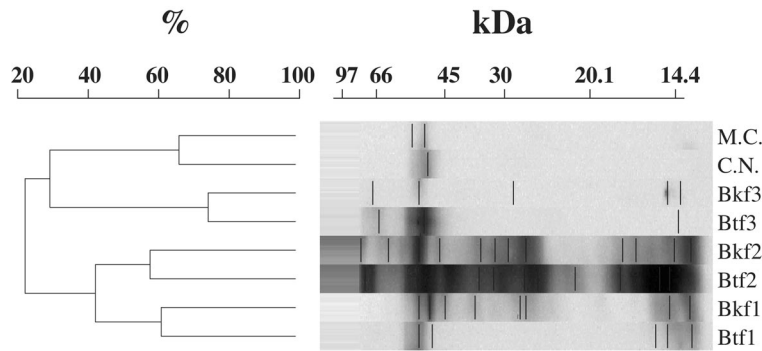


Fig. 2. SDS-PAGE IgE immunoblotting of extracts from mite corresponding to the different growth phases of *B. tropicalis* and *B. kulagini* cultures. Graphic representation of immunoblotting indicates the number and molecular weight (kilodaltons) of allergens (diversity) from extracts obtained at different growth phases (right) and the homology (percentage) between allergenic profiles of the extracts from each growth phase (left). Btf1, *B. tropicalis* extract from 15-d culture. Btf2, *B. tropicalis* extract from 120-d culture. Btf3, *B. tropicalis* extract from 160-d culture. Bkf1, *B. kulagini* extract from 15-d culture. Bkf2, *B. kulagini* extract from 100-d culture. Bkf3: *B. kulagini* extract from 160-d culture. MC, culture medium extract incubated with a positive serum. CN, *B. tropicalis* extract from 120-d culture, incubated with a negative serum.

kDa). *B. kulagini* also showed similar patterns in extracts from cultures incubated from 30 to 100 d (82% homology), revealing at least 11 different components (molecular weight range 100–14 kDa). The profiles of both mites showed maximum changes during the drop stage in agreement with the final increase of protein content measurements.

SDS-PAGE immunoblotting of extracts from mite cultures corresponding to different growth phases described is shown in Fig. 2. Note IgE binding proteins show maximum expression in extracts from cultures at the end of the exponential growth curve. Extracts from latent phase or drop stage revealed very scarce IgE binding proteins, although their protein profiles are considerable.

The kinetics of the concentration of Blo t 5 major allergen in culture extracts is represented in Fig. 3a and b. Concentrations of Blo t 5 demonstrated in extracts obtained from cultures of *B. kulagini* were higher than those obtained from cultures of *B. tropicalis* independently of growth phase of culture. Maximum growth also was associated to maximum Blo t 5 concentrations. At this point, the concentrations of Blo t 5 in *B. kulagini* were >10 times higher than in *B. tropicalis*.

Discussion

Under the conditions in this study, the two mites species showed a similar growth pattern, with three

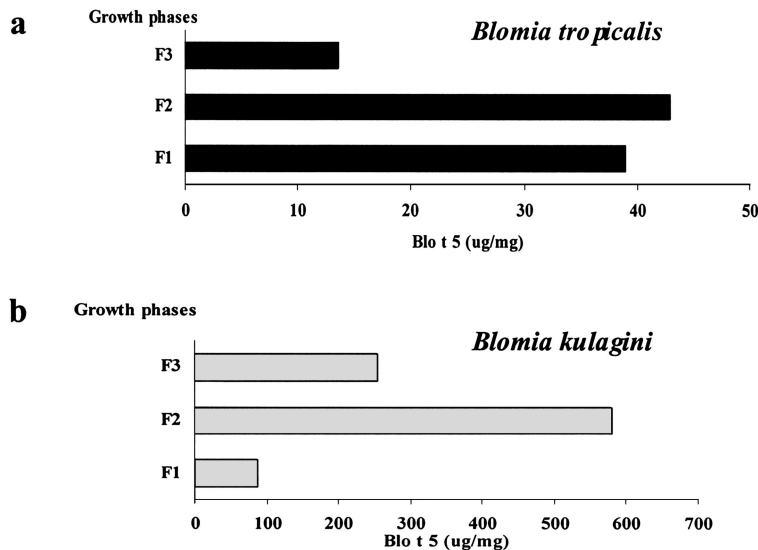


Fig. 3. Blo t 5 major allergen levels. (a) *B. tropicalis* culture extracts. F1, *B. tropicalis* extract from 15-d culture. F2, *B. tropicalis* extract from 120-d culture. F3, *B. tropicalis* extract from 160-d culture. (b) *B. kulagini* culture extracts. F1, *B. kulagini* extract from 15-d culture. F2, *B. kulagini* extract from 100-d culture. F3, *B. kulagini* extract from 160-d culture.

clearly distinct phases. An initial latency period was followed by an exponential growth period. Finally, after the rise in the maximum number of living mites, the cultures showed a drop stage characterized by a rapid decrease in the number of living mites.

The main differences between the curves of both species were observed in the time taken to reach the maximum number of living mites per gram of culture medium: 130 d for *B. tropicalis* and 58 for *B. kulagini*. No other studies have reported the dynamics of culture growth in *Blomia* spp., but in a previous study we reported similar curve patterns for house dust mites *Dermatophagoides pteronyssinus* (Trouessart, 1987) and *Dermatophagoides farinae* (Hughes, 1961) (Eraso et al. 1997), and we also found differences between the growth rates of the two species. *Blomia* spp. showed slower growth than *Dermatophagoides* spp., taking into account that culture methods used for all the mentioned species were the same.

SDS-PAGE patterns and their evaluation by image analysis showed an active change in protein profiles of both species over the course of the growth curves, and these profiles revealed maximum changes during the drop stage in agreement with the final increase of protein content measurements. Eraso et al. (1997) observed very similar behavior for *Dermatophagoides*, although they found shorter incubation times to reach the maximum growth. Yi et al. (1999), using fish food as culture medium, got the highest mite population in 4 wk, and concluded that in these conditions, *B. tropicalis* grew faster than *Dermatophagoides* spp. However, large differences in the composition of the culture media used by these authors make it difficult to compare the results with those obtained in this study.

More conclusive results are shown through immunochemical methods. Immunoblotting showed that the exponential growth phase of the mite cultures included the highest number of IgE binding components. The other culture phases, the latent and drop stages, showed little or no IgE binding components. According to these results, only the extracts from cultures in exponential phase are suitable to produce allergenic extracts with optimal quality. This fact coincides with the results obtained by Eraso et al. (1998) for house dust mites. Also, Andersen (1988) found that the allergenic activity of the extracts obtained from cultures of *D. pteronyssinus* at different growth stages correlated positively with the duration of the growth period.

The allergenic profile revealed by both species was very similar. IgE binding proteins with molecular weight in the range of 14–16 kDa were detected during all growth phases. It also should be noted that these components showed higher intensity development during the exponential growth period. Taking into account that Blo t 5 major allergen described for *B. tropicalis* has a molecular weight range compatible with the above-mentioned components, we measured the kinetics of Blo t 5 expression during the curve growth of *B. tropicalis* and *B. kulagini*. Results demonstrated that higher concentrations of Blo t 5 were found during the exponential phase, suggesting that

the 14–15-kDa components revealed by immunoblotting match up with group 5 of mite allergens. In a previous report, Eraso et al. (1997) also found the highest *Dermatophagoides* major allergens concentrations during the exponential phase of growth.

Blo t 5 was described as a major allergen of *B. tropicalis* showing 43% sequence homology to Der p 5 (Arruda et al. 1997). Johansson et al. (1997) suggested that *B. tropicalis* 14.5-kDa allergen is antigenically cross-reactive with *Lepidoglyphus destructor* (Schränk, 1781) allergen Lep d 2. Kuo et al. (2003) and Chew et al. (1999) demonstrated low or moderate levels of IgE cross-reactivity between Blo t 5 and Der p 5. No data have been reported about the presence of group 5 mite allergens in *B. kulagini*. Results obtained in this study demonstrate the expression of mite allergen belonging to group 5 by *B. kulagini* (Blo k 5) cross-reacting with its homologous in *B. tropicalis*.

The production of allergenic extracts to be used for diagnostic and therapeutic purposes must be carried out under excellent control. Exhaustive vigilance in the first step of extract production (mite cultures) is important to obtain reagents containing relevant allergens.

Acknowledgments

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