

Expression of the house dust mite allergen Der p 2 in the baker's yeast *Saccharomyces cerevisiae*

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Summary

Background and Results The major house dust mite allergen Der p 2 was expressed as a recombinant mature protein in the baker's yeast *Saccharomyces cerevisiae*. The yeast produces the protein fused to the invertase signal peptide, leading to the secretion of Der p 2 as a soluble protein into the culture medium. The signal peptide is hereby cleaved off, resulting in a mature allergen. In this system Der p 2 was produced in $7.6 (\pm 2.9)$ mg/L growth culture. Purification of the recombinant allergen was achieved by a single gel filtration step, resulting in a purity $\geq 95\%$. The yeast-derived Der p 2 was almost indistinguishable from natural Der p 2 with respect to IgE-reactivity and binding to the majority of Der p 2 specific MoAbs — as was shown in RAST analysis ($n = 168$) and a sandwich ELISA and RIA analysis, respectively. Recombinant and natural Der p 2 also showed similar biological activity in histamine release assays ($n = 4$).

Conclusion An expression system for Der p 2 was developed that enables the production of a soluble allergen in the culture supernatant with immunological characteristics similar to the natural allergen. In addition, yeast offers the advantage of the absence of endotoxin in comparison to *E. coli*. This might facilitate acceptance of recombinant allergens for *in vivo* applications as immunotherapy or skin-prick testing.

Keywords: baker's yeast, IgE, monoclonal antibodies, recombinant Der p 2, *Saccharomyces cerevisiae*

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Introduction

Allergens from the house dust mite *Dermatophagoides* spp. are among the most ubiquitous allergens on a world-wide basis. There is strong epidemiological evidence that sensitization and exposure to mite allergens is a highly significant risk factor for the development of asthma [1]. For the house dust mite *Dermatophagoides pteronyssinus* a number of allergens have been reported. The cDNA sequences of

several molecules are known [2]. In our study we focused on the group 2 allergen for several reasons. Studies in our laboratory indicate that this major allergen is as important as Der p 1 [3]. Der p 2 is a relatively small protein, that is not glycosylated and is easily detectable on immuno-blot (in contrast to Der p 1). Furthermore, Der p 2 is monomeric and successful expression in *E. coli* has been described [4].

The cDNA coding for the major allergen Der p 2 has been isolated [5]. It is a protein consisting of 129 amino acid residues with a molecular mass of 14 kDa. It is devoid of N-glycosylation sites and shows homology to the group 2 allergen from *D. farinae* [6] and a mammalian epididymous protein [7]. A role of Der p 2 as lysozyme has been

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suggested [8–10], but was so far not confirmed by experimental data. The availability of the cDNA enables the expression in microorganisms. Such a recombinant allergen can be used in studies on B-cell epitopes or T-cell reactivity. Furthermore, the recombinant allergen might be applicable in diagnostics and immunotherapy.

Der p 2 was initially expressed in *E. coli*, as a fusion protein with glutathion-S-transferase. Although this recombinant Der p 2 showed good reactivity with human IgE and a Der p 2 specific MoAb, not all Der p 2 reactive sera showed reactivity to the recombinant allergen [4]. This epitope deficiency might be caused by improper folding.

In this study Der p 2 was expressed in the baker's yeast *Saccharomyces cerevisiae*. The allergen is secreted by the yeast into the growth medium as a soluble protein, allowing isolation without denaturing steps. The yeast derived Der p 2 was compared with its natural counterpart with regard to reactivity with IgE and monoclonal antibodies.

Materials and methods

Sera

Sera of mite allergic patients ($n = 168$) were selected on ground of IgE reactivity with mite Der p 2 (RAST values ≥ 0.3 RAST Units IgE).

Monoclonal antibodies

The anti-Der p 2 monoclonal antibodies used were α DpX [3], 10E11, 5H7, 6H5, 8E5, 8H7, 7D6, 10G7, 10H7 and 10C1, obtained after immunization of BALB/b mice with a low-molecular weight fraction from mite whole body extract < 60 kDa [Van Leeuwen and Aalberse, unpublished data], 2B12B3, 4G7F1, 1E12E6, 1F9C6, 3G5A2, 1D8A6 [11], 6D6, 4E5 and 7A1 [12]. For the RAST, ascites or culture supernatants were used. mAbs 10E11 (culture supernatant) and α DpX (ascites) were protein-A purified. Purified α DpX was biotinylated, 1.2 mg MoAb/0.8 mg NHS-LC-biotine (Pierce, Rockford, Ill, USA).

Natural Der p 2

As source of natural Der p 2, a 2% extract of *D. pteronyssinus* whole mite bodies (Commonwealth Serum Laboratories, CSL, Parkville, Australia) was prepared in PBS/0.1% Tween-20 (PBS-T). Extraction time was 5 h. Bodies were removed by filtration. The group 2 allergen was affinity purified from this extract with the MoAb α DpX [3].

Molecular cloning

E. coli strains DH5 α [13] and JM110 [14] were used for the

amplification of plasmids. The *E. coli* strains were cultivated in 2YT medium (1.6% trypton, 1% yeast extract, 0.5% NaCl) at 37 °C, if necessary supplemented with 100 μ g ampicillin per mL.

General recombinant DNA techniques were performed as described by Maniatis [15]. Transformants were selected on 2YT (1.7% agar) plates containing 100 μ g/mL ampicillin. Recombinant clones were identified by restriction enzyme analysis and sequence analysis was performed by the chain termination method [16].

DNA modifying enzymes were purchased from Pharmacia Biotech (Uppsala, Sweden) or Gibco (Paisley, UK). For PCR, Vent polymerase (New England Biolabs Inc., Beverly, MA, USA) was used. DNA oligonucleotides were purchased from Pharmacia (Roosendaal, The Netherlands).

Yeast expression vectors

pSY1 [17,18] is a yeast episomal plasmid (YEp) of 7854 basepairs (bp). It contains the following elements: the ampicillin resistance gene (Ap), the origin of replication (ori) for selection and replication in *E. coli*, the LEU2 gene and a part of the yeast 2 μ m circle plasmid (2 μ m) for selection and replication in yeast. Since the vector was designed for the secretion of α -galactosidase, it contains a sequence coding for the fusion of the invertase signal peptide and the α -galactosidase (inv/ α -gal). Transcription of the hybrid gene is under control of the GAL7 promoter (GAL7pr) and is terminated by the phosphoglycerate kinase (PGK) terminator (PGKt). The location of the restriction sites for Eag 1 and Hind 3 is indicated (Fig. 1a).

pSY13 [19] is equal to pSY1, but contains the constitutive PGK promoter instead of the GAL7 promoter.

pUR2779 is a high copy integrating plasmid, based on pMIRY2 (plasmid for Multiple Integration into Ribosomal DNA in Yeast) and possesses a stretch of ribosomal DNA (rDNA) and a deficient LEU2 marker [20,21].

Production of the recombinant Der p 2 (recDer p 2)

Yeast strain *S. cerevisiae* BJ 1991 (*MAT α* , *leu2-1*, *trp1-1*, *ura 3-52*, *prb1-1122*, *pep4-3*) [22] was transformed by means of PEG/bicine method [23] with the appropriate plasmid, transformants were selected on minimal plates (0.67% Yeast Nitrogen Base (YNB) without amino-acids, 2% glucose, 20 μ g/mL uracil, 20 μ g/mL tryptophane and 2.2% agar). The transformants were grown for 40 h in minimal medium (as minimal plates, minus agar) and subsequently the yeast culture was diluted (10 times) in YPGal (1% yeast extract, 2% bacto-peptone, 4% galactose), YPGlu (glucose instead of galactose) or MM-Gal (as minimal medium, 4% galactose instead of 2% glucose), all at 30 °C. After 40 h or one week growth the

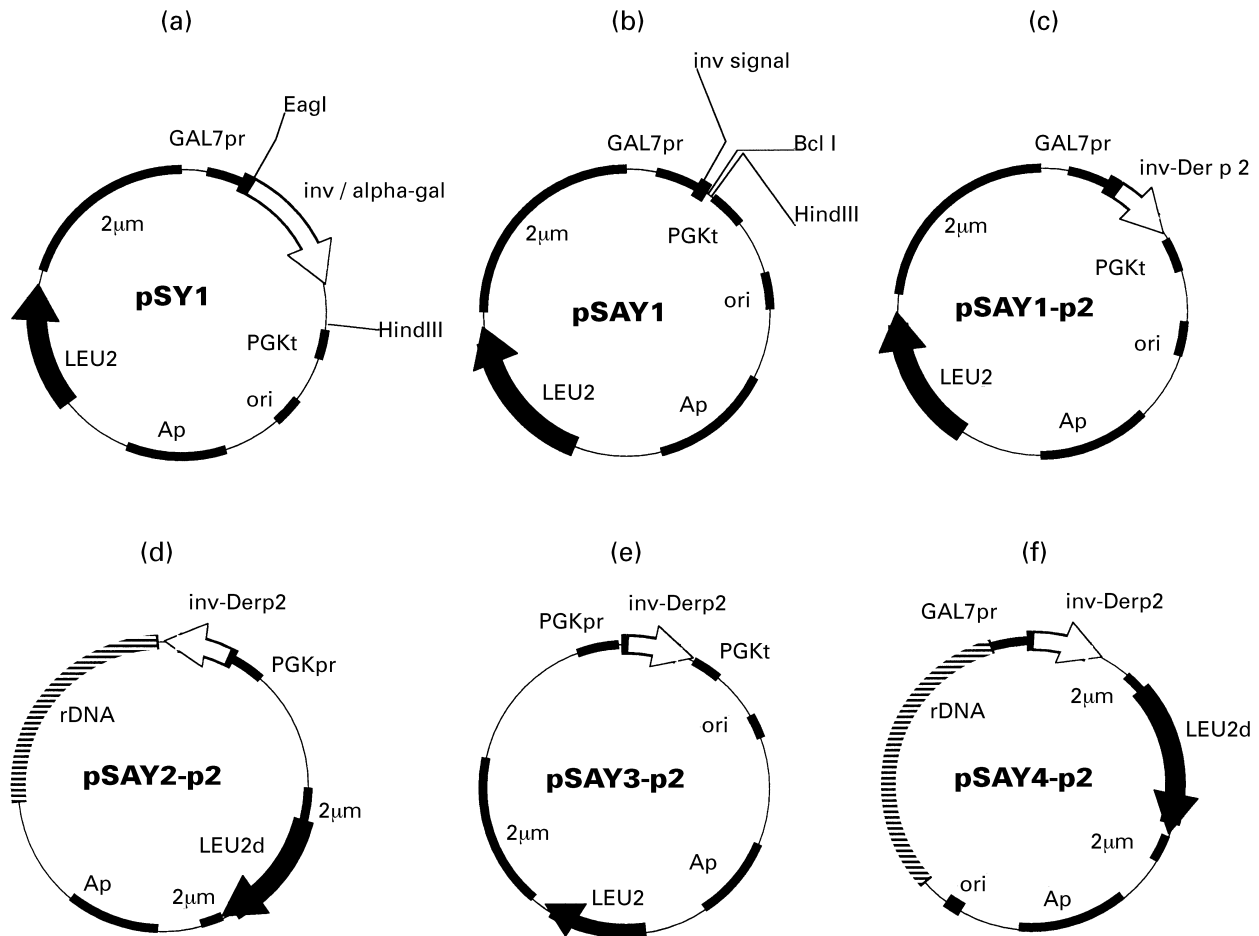


Fig. 1. The yeast expression plasmids (a) pSY1 the location of the restriction sites for *Eag* I and *Hin* d III are shown; (b) pSAY1 is equal to pSY1, α -galactosidase cDNA is replaced by a polylinker; (c) pSAY1-p2 contains the Der p 2 cDNA fused to the invertase signal peptide; (d) pSAY2-p2 is a hci plasmid with PGK promoter; (e) pSAY3-p2 is a YE_p, equals pSAY4-p2, but contains the PGK – in stead of GAL7 promoter; (f) pSAY4-p2 is a hci plasmid with the GAL7 promoter.

cells were spun down (20 min, 10.000 r.p.m.) and the growth medium was further analysed. The pSAY2,3-p2 transformants were grown in medium containing glucose instead of galactose.

Purification of Der p 2 from growth medium

Growth culture (500 mL) was concentrated on an Amicon ultrafiltration cell over a diaflo Ultrafiltration membrane YM10 (Amicon Corp, Danvers, MA, USA) to a volume of 10 mL. One millilitre of concentrate was applied to a Superdex 75 HR10/30 column (Pharmacia Biotech, Uppsala, Sweden) with a bed-volume of 23 mL, equilibrated in 0.05 M NaPO₄/0.5 M NaCl/pH = 7. The flow rate was 0.5 mL/min. Der p 2 eluted between 29 and 36 min (14.5 and 18 mL). Purity was judged by means of SDS-PAGE/silver-staining.

Protein measurements

Of the purified allergen, the protein concentration was measured with Pierce BCA Protein Assay Reagent (Pierce, Rockford, Ill, USA) at 37°C, following the manufacturer's instructions. BSA was used as protein standard.

N-terminal sequence analysis of the recombinant allergen

Purified rec Der p 2 was electrophoresed on a 15% Tricine-SDS PAGE [24], followed by electroblotting to Immobilon-P transfer polyvinylidene difluoride (PVDF) membrane (Millipore Corporation, Bedford, MA, USA). The sample was analysed by means of automatic Edman degradation (Applied Biosystems/Perkin Elmer Procise 494).

Der p 2 two-site ELISA

ELISA plates (Nunc, Roskilde, Denmark) were coated with MoAb 10E11 (1 µg/mL) overnight in 0.1 M NaHCO₃. Subsequently, the plates were coated with 0.1% BSA in 0.1 M NaHCO₃ for 30 min. Yeast derived culture supernatants or natural mite whole body extracts were incubated for 1 h in PBS/0.3% BSA/0.1% Tween-20 (PBS-AT). Incubation took place with biotinylated MoAb-αdpX (3 µg/mL in PBS/0.2% gelatine/0.02% Tween-20/0.1% BSA) for 1 h, followed by incubation with streptavidin-HRP [RPN 1231, concentration not provided] (Amersham, UK) in PBS 1:1000, after which staining took place with TMB. Between the incubation steps the plates were washed with PBS/0.01% Tween-20. As standards purified *rec* or natural Der p 2 with a known protein content were used.

RAST

Concentrated (50 x) growth (minimal) medium (about 300 µL), containing 50 µg Der p 2, was coupled to 300 mg CNBr-activated Sepharose-4B (Pharmacia Biotech, Uppsala, Sweden) according to the producer's manual. The Sepharose was suspended in 150 mL of PBS-AT. According to the same procedure, 300 µL concentrated (50 x) pSY1 growth medium, 1 mL 2% CSL mite whole body extract were coupled to 300 mg Sepharose. The pSY1 growth culture was used as a negative control for the yeast derived allergens. As negative control for the mite Sepharose, MoAbs were tested on glycine-Sepharose.

In a RAST, 500 µL (1 mg) Sepharose in PBS-AT was incubated with 50 µL human serum or MoAb over-night end-over-end, at room temperature. After washing five times with PBS-T, a ¹²⁵I radiolabelled antibody directed to either mouse-IgG or human IgE was added. After incubation over night with end-over-end rotation at room temperature, the Sepharose was washed four times with PBS-T and the bound radioactivity was measured, and percentage binding of total counts was calculated. Subsequently, results were expressed as arbitrary RAST-Units (RU)/mL. For calculation of RUs, a standard curve was used of several dilutions of a serum with known total IgE content incubated with Sepharose-coupled sheep antibodies against human IgE. RUs were calculated by using Slide Write Plus 6.0.

Indirect RAST

One millilitre MoAb αDpX containing ascites was coupled to 300 mg CNBr-activated Sepharose-4B (Pharmacia Biotech, Uppsala, Sweden) according to the producer's manual. The Sepharose was suspended in PBS-AT. Then 200 µL 2% CSL whole body mite-extract or 40 µL concentrated (50 x) yeast growth medium (both containing 6 µg

Der p 2) was incubated with 100 mg MoAb-Sepharose, in 50 mL PBS-AT overnight end-over-end. After washing five times with PBS-AT, the Sepharose was suspended in 50 mL PBS-AT. This solid phase was further used as described for the RAST.

Histamine release

Red blood cells were lysed by incubating 10 mL of blood with 40 mL of 155 mM NH₄Cl/10 mM KHCO₃/0.1 mM EDTA, pH = 7.2 for 5' at 4°C. After centrifugation a second lysis was performed in the same buffer. Cells were washed once with 0.9% NaCl, and then resuspended in 2.6 mL HEPES-medium (20 mM HEPES/132 mM NaCl/6 mM KCl/1.2 mM K₂HPO₄/1 mM MgSO₄/1 µM CaCl₂/5.5 mM glucose/0.5% HSA, pH = 7.2). For the release 250 µL of cell suspension (1–2% basophils) was incubated with 50 µL of allergen (tenfold dilutions were prepared) at 37°C under gentle shaking. Histamine content in the supernatant of the samples was measured with an automated fluorometric method [25]. Histamine release was expressed as percentage of the total histamine content of 250 µL of cell suspension, released by perchloric acid precipitation. Percentages spontaneous release (identical incubation without allergen) were subtracted.

At some occasions, basophils were isolated, by several centrifugation steps resulting in 2–5% basophils as described before [26]. Subsequently, basophils were stripped from their IgE by lactic-acid treatment followed by resensitization with IgE from human sera.

Results

Construction of expression plasmids

pSAY1

The pSY1 (Fig. 1a) was modified for the expression of Der p 2 as follows. By means of a PCR on the invertase signal in pSY1 and several subcloning steps (not shown), the α-galactosidase cDNA was replaced by a polylinker consisting of a *Bcl* I site, followed by the *Kpn* I – *Hin* d III fragment out of the polylinker of pUC19 [14]. This resulted in the plasmid pSAY1 (plasmid for the secretion of allergen from yeast). See Fig. 1(b).

pSAY1-p2

In order to isolate the Der p 2 cDNA as a *Bcl* I – *Hin* d III fragment, the 596 bp *Eco* R I fragment from pUC9-p2 [5] was isolated and ligated into pUC19, resulting in the plasmid pUC19-p2 (not shown). From this construct the cDNA was isolated as a 519 bp *Bcl* I – *Hin* d III fragment

and ligated into pSAY 1 digested with *Bcl* I and *Hin* d III, resulting in the construct pSAY1-p2 (Fig. 1c).

pSAY2,3,4-p2

To improve the production level of the allergen the plasmid pSAY4-p2 was constructed (Fig. 1f). This expression plasmid is derived from the high copy integrating (*hci*) plasmid pUR2779 [27]. Also two plasmids with a pGK promoter were developed, a *hci* plasmid pSAY3-p2 (Fig. 1e), derived from pSY13 and a YEp pSAY2-p2 (Fig. 1d). This plasmid is derived from pSAY4-p2, the GAL7 promoter is replaced by the PGK promoter.

Expression of the allergen from different plasmids

The four plasmids were transformed into yeast and transformants were grown in YPGal or YPGlu for 40 h. The concentration of Der p 2 in growth medium was determined in a two site ELISA (Table 1). The average concentration in case of pSAY1-p2 was $7.6 (\pm 2.9)$ mg/L (nine different colonies from one single transformation). In other transformations comparable yields were achieved.

For further studies the pSAY1-p2 was used, in MMgal, after a week growth, giving $\approx 50\%$ of the yield in YPGal after 40 h, while the protein background is less.

Purification of the allergen from growth medium and sequence analyses

A gel filtration was carried out to purify Der p 2. The fractions containing Der p 2 were pooled and tested for purity by means of SDS-PAGE, followed by silver staining. Purity was at least 95% (Fig. 2).

N-terminal sequence analysis of rec Der p 2 was carried out. The predicted sequence [5] was confirmed by analysis of the first seven amino acid residues: DQVDVKD. Thus, the invertase-Der p 2 is correctly processed by the yeast,

Table 1. Expression of Der p 2 from different plasmids

Plasmid	Type	Promotor	[Der p 2]	mg/L
pSAY1-p2	YEp	GAL7	7.6 ± 2.9	(<i>n</i> = 9)
pSAY2-p2	<i>hci</i>	PGK	15.7 ± 3.6	(<i>n</i> = 6)
pSAY3-p2	YEp	PGK	3.6 ± 1.5	(<i>n</i> = 9)
pSAY4-p2	<i>hci</i>	GAL7	32.2 ± 7.6	(<i>n</i> = 5)

From each plasmid a number of yeast transformants were grown in YPGa1, or YPGlu and the concentration of Der p 2 was measured by ELISA. The standard deviation and the number of transformants (between brackets) are given.

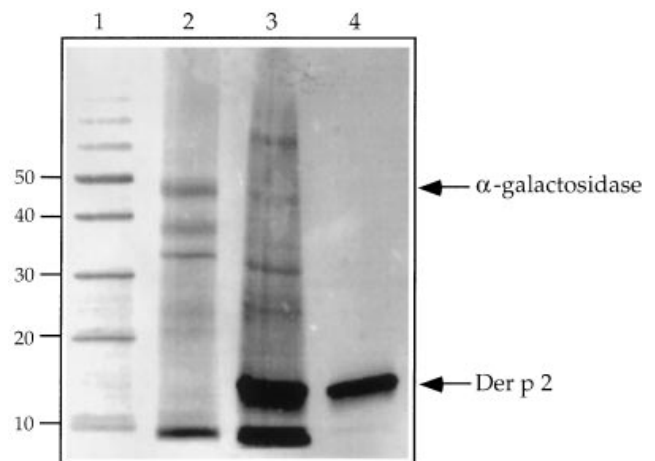


Fig. 2. Expression and purification of Der p 2 SDS-PAGE of protein 10 kDa ladder (1) growth medium from a pSY1 (2) and a pSAY1-p2 (3) transformant and purified Der p 2 from the growth medium (4). The gel was silver-stained.

resulting in the secretion of a mature allergen molecule into the growth culture supernatant.

Reactivity with MoAbs

Purified *rec* Der p 2 and affinity purified natural Der p 2 were compared in a two-site ELISA with Der p 2 specific MoAbs 10E11 and α DpX. Both allergens demonstrated similar reactivity (Fig. 3).

Furthermore, a panel of 18 MoAbs was tested on mite and *rec* Der p 2 in a direct RAST (Fig. 4a). Four out of

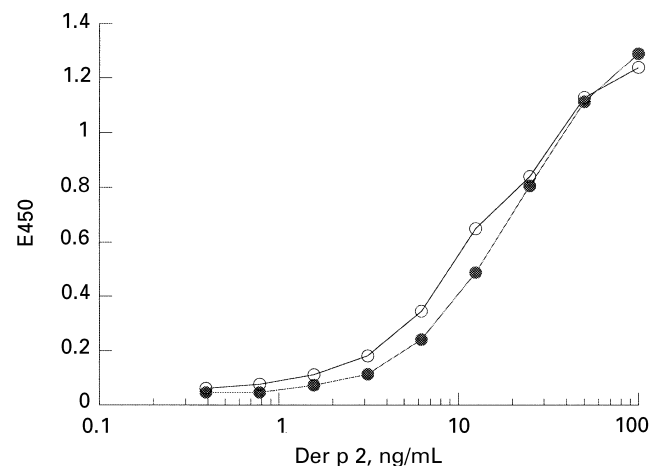


Fig. 3. Reactivity of mite — and *rec* Der p 2 in a two-site ELISA. A comparison of the reactivity of natural and *rec* Der p 2 in a two-site ELISA is shown. Protein concentrations were determined by means of Pierce. m indicates the values for mite Der p 2, ● for *rec* Der p 2.

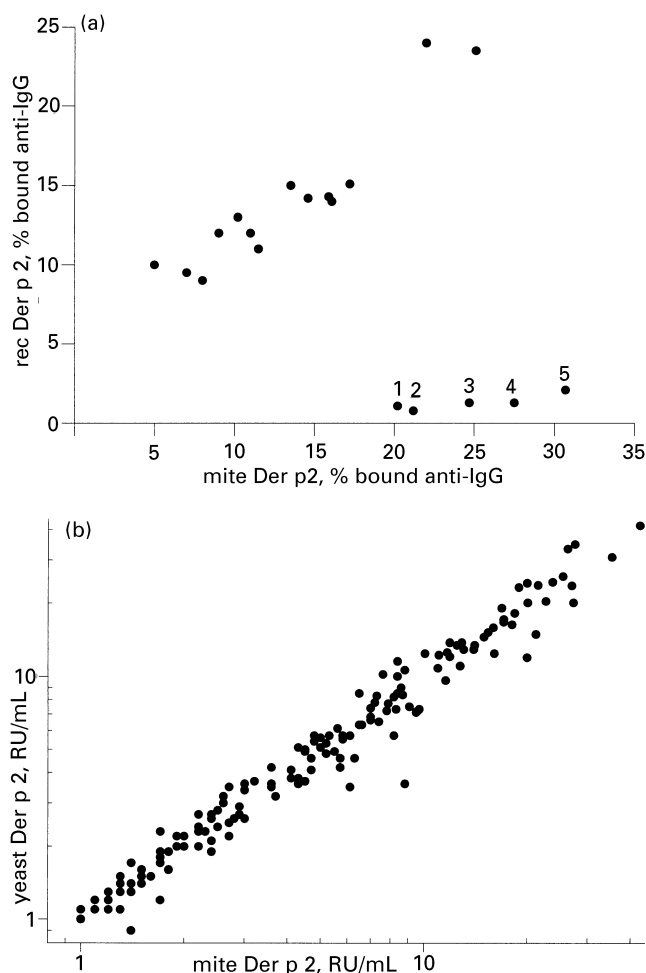


Fig. 4. Reactivity of mite and rec Der p 2 with MoAbs and IgE. (a) set of 18 MoAbs was tested in a RAST on mite and rec Der p 2. Dilutions of MoAbs were for culture supernatants 1:50 (10C1, 5H7, 6H5, 8E5, 8H7, 7D6, 10G7, 10H7, 10E11 and α Can f 1) or 1:10.000 (α DpX and 2B12B3), ascites (4G7F1, 1E12E6, 1F9C6, 6D6, 3G5A2, 4E5 and 7A1) 1:1.000. Results are given as percents binding of anti-IgG. (1)=10C1; (2)=1D8C4; (3)=4G7F1; (4)=1F9C6; (5)=1E12E6. (b) 168 house dust mite patients showing IgE binding to natural Der p 2 were tested in an indirect RAST with native as well as rec Der p 2. Results are expressed in RU/mL.

18 MoAbs were not reactive with the yeast derived group 2 allergen. These MoAbs were: 10C1 (1), 1D8C4 (2), 4G7F1 (3), 1F9C6 (4), 1E12E6 (5). The remaining 14 MoAbs showed similar reactivity with mite and rec Der p 2.

As negative control for the rec Der p 2, pSY1 growth culture (containing α -galactosidase) was tested, as negative control for the MoAbs, a MoAb directed towards the allergen Can f 1 was tested. All these RAST values were <2% (results not shown).

Table 2. Sera showing low reactivity towards rec Der p 2

Serum	Mite extract RAST Units	Yeast culture RAST Units	Ratio y/m
782006	6.1	3.5	0.6
782180	8.8	3.6	0.4
54	1.4	<0.3	<0.2
782703	20.0	11.9	0.6

From four sera with a significant lower reactivity towards rec Der p 2 the RAST units are listed in this table. The ratio of the two values is given in the last column.

IgE-binding properties

We selected 168 house dust mite patients showing IgE reactivity with mite Der p 2 in an indirect RAST. These patients were tested in an indirect RAST with rec Der p 2 (Fig. 4b). Linear regression analyses showed a close correlation of IgE reactivity with both allergens ($n=168$, $r=0.973$, $P=0.0001$), while the mean ratio of anti-rec Der p 2/anti-mite Der p 2 IgE was $1.001 (\pm 0.149)$. In case of four sera the ratio yeast/mite IgE units was <0.7 (Table 2). In one of these four sera — with low reactivity to mite Der p 2 — no reactivity towards rec Der p 2 was detected.

Biological activity: histamine release

The biological activity of the recombinant allergen was studied in a histamine release assay. The recombinant allergen was able to induce histamine release from basophils for all four patient tested. The ratio rec Der p 2/mite Der p 2 required to achieve optimal histamine release was approximately 0.5 for three patients and approximately 1 for one patient. The histamine release curves for two patients are represented (Fig. 5). As a negative control for the yeast derived allergen, pSY1 growth culture containing α -galactosidase was used.

Discussion

The bacterium *Escherichia coli* is the host organism frequently used for the production of recombinant allergens. However, the baker's yeast *Saccharomyces cerevisiae* might be preferred over *E. coli* for this purpose when *in vivo* applications — such as immunotherapy or skin-prick testing — are aimed. The reason is that *E. coli* produces toxic and pyrogenic cell wall components, whereas *S. cerevisiae* has, during long time use in food industry, proven to be non-pathogenic to man.

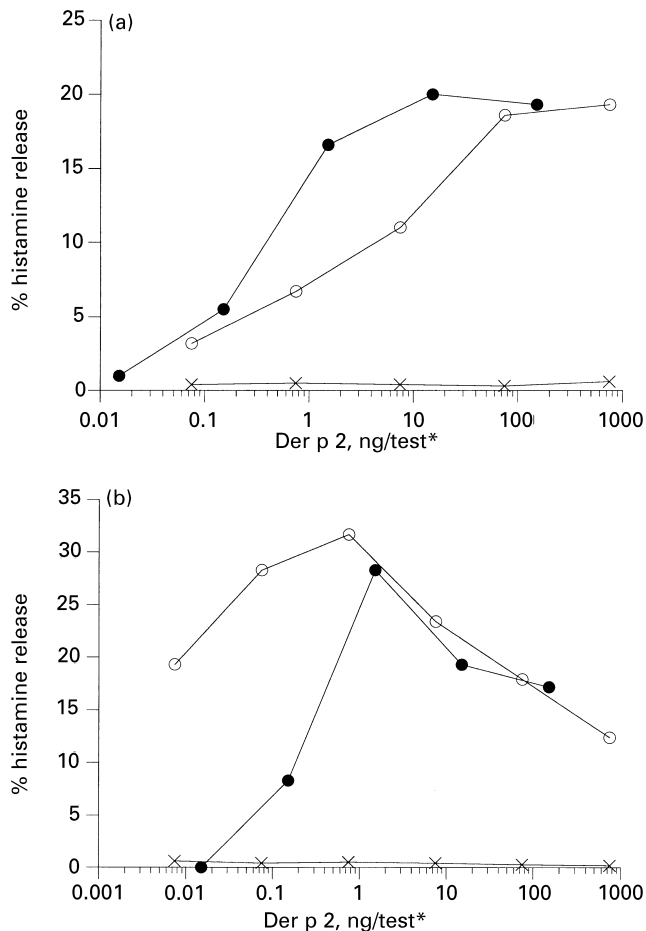


Fig. 5. Histamine release with *rec* Der p 2. White blood cells were isolated from peripheral blood from patient CV (a) and stripped basophils from a nonatopic donor were resensitized with IgE from serum 135 (b). These cells were tested in the histamine release assay with purified mite Der p 2 and Der p 2 containing yeast growth culture. Per cent released histamine is represented. The symbols used, are: ○ = mite Der p 2, ● = *rec* Der p 2, X = yeast derived α -galactosidase. *For α -galactosidase points were taken with protein-concentrations corresponding to the titration of Der p 2 containing growth culture.

In our study the expression vector pSY1 was modified for expression of allergens: pSAY1. The pSAY1-p2 transformants are secreting the Der p 2 allergen as a soluble protein into the growth medium. The protein can be purified by means of gel filtration. In this way no de- and re-naturing steps are required. This recombinant allergen molecule showed good Der p 2 reactivity with regard to histamine release and reactivity with several antibodies (IgE and MoAbs). In a study described with bacterial fusion protein GST-Der p 2, 2 out of 24 patient sera showing reactivity to natural Der p 2, did not react with this particular recombinant Der p 2 [4]. In our study 168 sera were tested, only one

serum (<1%) was unreactive. However, further analyses revealed that this serum showed a very weak reactivity with purified natural Der p 2 in a direct RAST. The IgE in the indirect RAST (with mite body extract) was mainly reactive with a component of about 12 kDa (results not shown, manuscript in preparation). This might be a breakdown product of the 14 kDa allergen, which is present in natural allergen preparations but not in the recombinant preparation. The lower reactivity of the other three sera with the *rec* Der p 2 might also be explained by the presence of the 12 kDa component.

We found four MoAbs that were not reactive with the *rec* Der p 2 derived from yeast. Further analyses however, revealed that these MoAbs were specific for one isogenetic variant, differing in one amino acid residue from the one we expressed in this study [28].

In order to improve the production level, hci Der p 2 expression plasmids were constructed. These plasmids are based on pMIRY2 [20,21]. This vector possesses a fragment of ribosomal DNA from *S. cerevisiae* for integrating into this chromosomal locus by homologous recombination and a deficient LEU 2 marker (which lacks a promoter sequence, resulting in a marked decrease in expression level). This plasmid is integrating into the yeast chromosome at a high copy number (150–200 copies per cell). pMIRY2-plasmids bearing a heterologous or homologous gene are giving rise to an increased production of recombinant protein, compared with low copy-vectors [20,21]. Indeed, by using the plasmid pSAY4-p2 we could demonstrate an increase in allergen-production by a factor of 4. For the production of Der p 2 this vector is very suitable. Since the transformation with h.c.i. plasmids takes two weeks, pSAY1-p2 might be preferred for a rapid isolation, for example in testing products from mutated constructs. Furthermore, plasmids with the constitutive PGK promoter were constructed. In this way the Der p 2 can be produced with the yeast growing on glucose instead of galactose. This might be required in certain circumstances, for example in case of *in vivo* labelling experiments.

In conclusion, we expressed the house dust mite group 2 allergen in the baker's yeast *S. cerevisiae*. This allergen can be isolated as a soluble protein, no denaturing steps are required. The yeast derived protein possesses a good Der p 2 reactivity, there is a good correlation between IgE reactivity with recombinant and natural Der p 2. Furthermore, the allergen is able to induce histamine release from basophils. The fact that this yeast is granted GRAS (Generally Recognized As Safe) facilitates *in vivo* applications, such as immunotherapy or skin-prick testing.

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References

- Sporik R, Platts-Mills TAE, Holgate ST, Cogswell JJ. Exposure to house dust mite allergen (Der p 1) and the development of asthma in childhood. *New Eng J Med* 1990; 323:502–7.
- Stewart GA, Thompson PJ. The biochemistry of common aeroallergens. *Clin Exp Allergy* 1996; 26:1020–44.
- Van der Zee JS, Van Swieten P, Jansen HM, Aalberse RC. Skin test and histamine release with P1-depleted *Dermatophagoides pteronyssinus* body extracts and purified P1. *J Allergy Clin Immunol* 1988; 81:884–96.
- Chua KY, Dilworth RJ, Thomas WR. Expression of *Dermatophagoides pteronyssinus* allergen Der p 2 in *Escherichia coli* and the binding studies with human IgE. *Int Arch Allergy Appl Immunol* 1990; 91:124–9.
- Chua KY, Doyle CR, Simpson RJ et al. Isolation of cDNA coding for the major mite allergen Der p 2 by IgE plaque immunoassay. *Int Arch Allergy Appl Immunol* 1990; 91:118–23.
- Thomas WR, Chua KY, Stewart GA et al. Analysis and expression of cDNA clones coding for house dust mite allergens. *Advances in the Biosciences* 1989; 74:139–47.
- Thomas WR, Chua KY. The major mite allergen Der p 2 - a secretion of the male mite reproductive tract? *Clin Exp Allergy* 1995; 25:667–9.
- Chapman M. Manipulating allergen genes. *Clin Exp Allergy* 1991; 21:155–6.
- Stewart GA, Birch CH, Thompson PJ. Do the group 2 allergens correspond to lysozyme? *J Allergy Clin Immunol* 1992; 90:141–2.
- Khlgatian SV, Perova NA. Allergens from *Dermatophagoides* dust mites: origin, antigenic and structural characteristics and therapeutic agents. *Biokhimiia* 1995; 60:218–37.
- Ovsyannikova IG, Vailes LD, Li Y, Heymann PW, Chapman MD. Monoclonal antibodies to group 2 *Dermatophagoides* spp allergens; murine immune response, epitope analysis and development of a two site ELISA. *J Allergy Clin Immunol* 1994; 94:537–46.
- Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE. Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989; 83:1055–67.
- Hanahan D. Studies on the transformation of *Escherichia coli* with plasmids. *J Mol Biol* 1983; 166:557–80.
- Yanisch-Perron C, Vieira J, Messing J. Improved M13 phage cloning vectors and host strains: nucleotide sequence of the M13 mp18 and pUC 19 vector. *Gene* 1985; 33:103–19.
- Maniatis T, Fritsch EF, Sambrook J. *Molecular cloning, a laboratory manual*. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York, 1982.
- Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain terminating inhibitors. *Proc Natl Acad Sci USA* 1977; 74:5463–7.
- Harmsen MM. Heterologous protein secretion by the yeast *Saccharomyces cerevisiae*. PhD thesis, 1995, Free University, Amsterdam.
- Harmsen MM, Langedijk AC, Van Tuinen E et al. Effect of a pmr 1 disruption and different signal sequences on the intracellular processing and secretion of *Cyamopsis tetragonoloba* alpha-galactosidase by *Saccharomyces cerevisiae*. *Gene* 1993; 125:115–23.
- Schreuder MP, Brekelmans S, Van den Ende H, Klis FM. Targetting of heterologous protein to the cell wall of *Saccharomyces cerevisiae*. *Yeast* 1993; 9:399–409.
- Lopes TS, Klootwijk J, Veenstra AE et al. High-copy-number integration into the ribosomal DNA of *Saccharomyces cerevisiae*: a new vector for high-level expression. *Gene* 1989; 79:199–206.
- Lopes TS. A novel ribosomal DNA vector for high expression of heterologous genes in yeast PhD thesis. Free University. 1990.
- Jones EW. Proteinase mutants of *Saccharomyces cerevisiae*. *Genetics* 1977; 85:23–33.
- Klebe RJ, Harris JV, Sharp ZD, Douglas MG. A general method for poly-ethylene-glycol-induced genetic transformation of bacteria and yeast. *Gene* 1983; 25:333–41.
- Schägger H, Von Jagow G. Tricine-Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis for the Separation of Proteins in the Range from 1 to 100 kDa. *Anal Biochem* 1987; 166:368–79.
- Siraganian RP. Refinements in the automated fluorometric histamine analysis system. *J Immunol Meth* 1975; 7:283–90.
- Pasmans SGMA, Witteman AM, Aalbers M et al. Variability of an IgE-dependent histamine-releasing activity in supernatants of human mononuclear cells. *Int Arch Allergy Appl Immunol* 1994; 103:44–52.
- Verbakel JMA. Heterologous gene expression in the yeast *Saccharomyces cerevisiae*. PhD thesis, 1991, Rijksuniversiteit Utrecht, The Netherlands.
- Hakkaart GAJ, Chapman MD, Aalberse RC, Van Ree R. Immune-reactivity of recombinant isoforms of the major house dust mite allergen Der p2. *Clin Exp Allergy* 1998; in press.