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Expression of Recombinant Hypoallergenic Sui p 2 in *Saccharomyces boulardii*, a Potential Prophylactic Agent for House Dust Mite Allergy

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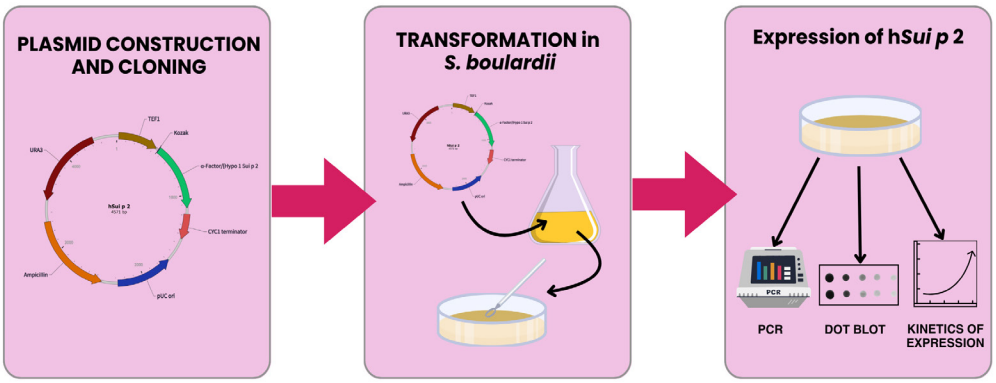
Graphical Abstract



Abstract

Recombinant hypoallergenic proteins have been explored for allergy immunotherapy and prophylaxis, while probiotics have demonstrated protective effects against allergic diseases. This study describes the expression of a hypoallergenic Sui p 2 allergen from the house dust mite (HDM) *Suidasia pontifica*, in the probiotic yeast *Saccharomyces boulardii*, in order to generate a potential prophylactic agent for HDM allergy. Modified sequence of the *Sui p 2* gene was cloned and expressed in *S. boulardii* (rSb) as a recombinant hypoallergen. The 378 bp *hSui p 2* sequence was inserted into the yeast plasmid pYIP-*hSui p 2*, which underwent digestion with StuI before transformation via high-efficiency LiAc/SS. Gel electrophoresis after StuI restriction enzyme digestion confirmed successful transformation, revealing a 4571 bp plasmid while Polymerase Chain Reaction confirmed the presence of *hSui p 2* insert integrated into the genome of Sb. Growth curve analysis indicated that both rSb exhibited a slower growth rate, a delayed peak, and a slightly lower biomass yield compared to the untransformed clone of *S. boulardii* (Sb). Furthermore, dot blot immunoassay using anti-His antibodies verified the successful expression of rSb. The expression of a recombinant hypoallergen Sui p 2 as an initial step in the development of a potential prophylactic agent for HDM allergy.

Keywords: recombinant Sui p 2; hypoallergen; *Saccharomyces boulardii*; immunotherapy

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Introduction

House dust mites (HDMs) are among the most common allergens, responsible for 60–90% of allergic cases [1]. Exposure to HDM allergens not only increases the likelihood of developing asthma but also worsens allergic symptoms [2]. The HDM species, *Suidasia pontifica* (Sp), also known as *Suidasia medanensis*, has been identified as a significant airborne allergen source linked to asthma episodes in household environments. This species is highly prevalent and found in approximately 80% of homes in tropical regions, highlighting its clinical and basic allergology importance [3,4,5]. Given the widespread occurrence of HDM allergies across developed and developing regions of the world, the development of innovative and targeted treatment strategies is crucial in mitigating allergic symptoms and improving patient outcomes.

Among the various HDM allergens, Sui p 2, a major allergen from *Suidasia pontifica*, has emerged as a potential target for allergen-specific immunotherapy (ASIT) and allergy prophylaxis. Engineering hypoallergenic variants of Sui p 2 could provide an effective and safer alternative for HDM allergy treatment. Additionally, probiotics have gained attention as a complementary approach in ASIT. Certain probiotic strains within the gut mucosa exhibit immunomodulatory effects by shifting cytokine production from a Th2-biased response to a Th1 profile, thereby mitigating allergic symptoms. *Saccharomyces boulardii* (Sb), a well-characterized probiotic yeast known for its ability to survive in harsh gut conditions, has been recognized as a promising candidate for vaccine delivery [6,7]. The expression of hypoallergens in Sb allows for proper protein folding and post-translational modifications, offering an innovative approach to ASIT and allergy prophylaxis [8].

In this study, we report the cloning and expression of a recombinant hypoallergenic protein, recombinant Sui p 2, in Sb. The Sui p 2 hypoallergen, contains modified IgE epitopes, was successfully expressed in *Saccharomyces boulardii*. The genetically engineered Sb is a potential agent for hypoallergenic vaccine development for HDM allergy, an important advancement in the development of safer and more effective therapeutics for ASIT and as a potential prophylactic agent.

Materials and methods

In silico designed hSui p 2. The Sui p 2 gene (GenBank with accession number KY449406) was used in a series of in silico analyses to characterize its protein structure. the signaling peptide was removed from the sequence, leaving a 378 bp long nucleotide sequence that translated into 126 amino acids, with a predicted molecular weight of 14.35 kDa. The sequence was then submitted to Alphafold2, Swiss model, and I-TASSER to generate a three-dimensional (3D) model [9]. Similarly, the model's quality was evaluated using PROCHECK [10] and ERRAT [11] through the SAVES v6.0 server [12]. Structural visualization was performed with ChimeraX ver 10.12 software.

In the Sui p 2 hypoallergen design, T-cell epitopes were excluded from the B-cell epitope-based hypoallergen to minimize T-cell epitope-mediated late-phase side effects [13,14]. T-cell epitopes were predicted using TepiTool (http://tools.iedb.org/tepitool/) [15], targeting the 26 most frequent human class II alleles from HLA-DR, HLA-DQ, and HLA-DP with a percentile rank ≤ 10. The final T-cell epitope was selected from those containing two or more MHC-II alleles.

B-cell linear and conformational epitopes were identified using several computational prediction methods. The sequence was submitted to the ElliPro server (http://tools.iedb.org/ellipro/) [16] with a score threshold >0.5. Bepipred 2.0 (http://tools.iedb.org/bcell/) [17] and Graphbepi [18] were used to predict B-cell epitopes, with a threshold of 0.5. Final linear B-cell epitopes were selected from sequences shared by two or more prediction methods. Conformational epitopes were identified based on the 3D structure of the protein, as they significantly contributed to allergenicity in inhalant allergens [19]. Two AI methods, Seppa 3.0 (http://www.badd-cao.net/seppa3/index.html) [20] and SEMA (http://sema.airi.net) [21], were used to predict key residues for discontinuous B-cell epitopes, with the Seppa 3.0 threshold set at 0.064 and the SEMA value at more than 1.1. Similarly, toxicity of the hypoallergen was also tested using Toxin Pred (https://webs.iiitd.edu.in/raghava/toxinpred/index.html). Subsequently, all results were integrated to create a hypoallergenic Sui p 2, in which allergenic epitopes and cystine residues were substituted with alanine to reduce basophil activation and IgE binding while increasing IgG secretion [22].

The pYIP-hSui p 2 Construct. The 378 bp hypoallergenic Sui p 2 was constructed and cloned into a yeast-integrating plasmid (pYIP) generating a 4571 bp pYIP-hSuip2 construct. This plasmid vector included an endogenous URA3 gene, enabling yeast survival without uracil and uridine, and facilitated plasmid integration into the yeast genome through homologous recombination, which involved the StuI restriction enzyme site for linearization [23,24]. A strong yeast promoter region, TEF1, was also included [25]. Additionally, a Kozak sequence was incorporated to facilitate the translation initiation of the ATG start codon, essential in eukaryotic translation [26]. The construct was tagged with an alpha-factor signal peptide at the N-terminus, necessary for recombinant protein secretion, and a 6-histidine tag at the C-terminus. It also contained a CYC1 terminator region, allowing transcription termination and polyadenylation of mRNA transcribed by RNA polymerase II in yeast [27]. Moreover, the plasmid included a pUC origin site for plasmid replication and regulation of high-copy plasmid numbers and an ampicillin resistance gene for selection in *E. coli* [28]. Plasmid construction, cloning, and transformation in *E. coli* was facilitated by VectorBuilder (https://en.vectorbuilder.com/).

Plasmid Isolation. T The hSui p 2 plasmid was isolated from *E. coli* using the ZymoPURE™ Plasmid Miniprep Kit. A 0.5 mL aliquot of *E. coli* stock was centrifuged in a 1.5 mL tube for 15-20 seconds, and the supernatant was discarded. The bacterial cell pellet was resuspended in 250 µL of cold ZymoPURE™ P1 (Red) by vortexing or pipetting. A 250 µL of ZymoPURE™ P2 (Blue) was then added and mixed by gently inverting the tube 8-10 times, allowing it to sit at room temperature for 3 minutes until the solution became clear, purple, and viscous. Subsequently, 250 µL of ZymoPURE™ P3 (Yellow) was added and mixed by inversion, with the tube inverted 5 times once the sample turned completely yellow. The neutralized lysate was centrifuged for 5 minutes at 16,000 x g, and 600 µL of the supernatant was transferred to a clean 1.5 mL tube. Afterwards, 260 µL of ZymoPURE™ Binding Buffer was added to the cleared lysate and mixed by vortexing for 15 seconds. The mixture was transferred to a Zymo-Spin™ II-PX Column placed in a collection tube, incubated at room temperature for 1 minute, and then centrifuged at ≥ 10,000 x g for 1 minute, discarding the flow-through. Then, 800 µL of ZymoPURE™ Wash 1 was added to the column and centrifuged at ≥ 10,000 x g for 1 minute, discarding the flow-through. This was followed by adding 800 µL of ZymoPURE™ Wash 2, centrifuging at ≥ 10,000 x g for 1 minute, and discarding the flow-through. Another 200 µL of ZymoPURE™ Wash 2 was added and centrifuged at ≥ 10,000 x g for 1 minute, discarding the flow-through between steps. The column was centrifuged again at ≥ 10,000 x g for 1 minute to remove any residual wash buffer. Lastly, the column was transferred to a clean 1.5 mL tube, and 25 µL of ZymoPURE™ Elution Buffer was added directly to the column matrix. The plasmid DNA was incubated at room temperature for 2 minutes and then centrifuged at ≥ 10,000 x g for 1 minute. The eluted plasmid DNA was stored at ≤ -20°C. Isolated plasmid was quantified and purity was determined using UV-Vis spectrophotometry (BMG LABTECH). Absorbance measurements were taken at 260 nm and 280 nm to evaluate DNA concentration and protein contamination, respectively.

Polymerase Chain Reaction. Polymerase chain reaction (PCR) was performed for the confirmation of the isolated hSui p 2 plasmid. A 20 µL PCR mastermix was prepared using 3 µL 10µM DNA Isolate, 2 µL Taq PCR mastermix (Solarbio, Beijing, China), 14 µL of nuclease-free water, and 1 uL of StuI restriction enzyme (New England Biolabs, Inc.). The PCR tubes were loaded into a Mini16 Thermal Cycler (MiniPCR, USA) with the following parameters: initial denaturation set at 94 °C, denaturation at 90 °C, annealing at 55 °C, elongation at 72°C, and final elongation at 72°C, each stage set for 60 seconds, with a total of 30 cycles. PCR products were run on a 1% agarose gel electrophoresis for 1 hour at 25V. A 1 kb Plus DNA Ladder (Invitrogen, Thermo Fisher Scientific) was used to determine the molecular weights.

Transformation in Sb. The lithium acetate transformation method was used to transform a linearized vector construct containing StuI (New England Biolabs, Inc.) into Sb. Specifically, a 20 mL overnight liquid yeast culture with an absorbance value of 0.5 at OD600 was obtained by centrifugation. A transformation reaction mixture consisting of 240 µL of Polyethylene Glycol (PEG), 50 µL of pre-boiled salmon sperm, 14 µL of autoclaved water, 20 µL of StuI linearized plasmid, and 36 µL of LiAc was combined, vortexed, and then incubated for 30 minutes in a water bath at 42°C.

Subsequently, 40 µL of Dimethylsulfoxide (DMSO) was added to the transformation mixture, followed by another 30-minute incubation in a water bath at 42°C. Subsequently, cells were collected by centrifugation and resuspended in 100 µL of sterile water for complete homogenization using a vortex. Yeast cells were then plated onto an SD-URA agar plate and cultured at 30°C for 3 to 5 days. Colonies of successfully transfected *Saccharomyces boulardii*, known as rSb, were restreaked onto a different plate. Similarly, rSb glycerol stocks were prepared by pipetting 800 µL of rSb in SD-URA liquid culture and 200 µL glycerol into a cryogenic tube. The rSb glycerol stock was stored at an ideal refrigerated temperature of 2-4°C for subsequent analysis.

Genomic DNA Isolation. The Genomic DNA from a transformed Sb was isolated using the YeastarTM Genomic DNA Kit (Zymo Research, USA) to confirm the positive integration of the hSui p 2 gene. A 1 mL rSb clone culture containing approximately 1.5x10^7 cells was collected by centrifugation for 2 minutes at 500 g. The cells were lysed with 120 µL of YD Digestion Buffer and 5 µL of R-Zymolyase (RNase A + Zymolyase). The pellet was resuspended, vortexed, and incubated at 37°C for 60 minutes. Subsequently, 120 µL of YD Lysis Buffer was added and vortexed for 10-20 seconds. Then, 250 µL of chloroform was added and mixed thoroughly for 60 seconds, followed by centrifugation at 10,000 rpm for 2 minutes. The top layer was then transferred to a Zymo-spin III Column, centrifuged at 10,000 rpm for 1 minute, and washed twice using 300 µL of DNA wash buffer with centrifugation for 1 minute. The DNA was then eluted with 60 µL of sterile water and stored at -20°C until usage.

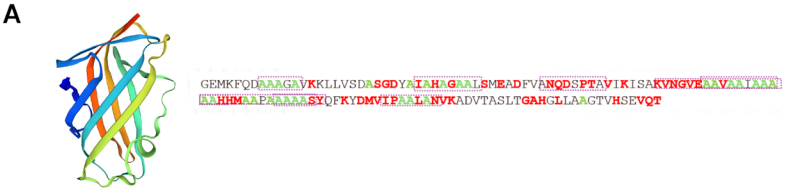
Growth Kinetics Analysis. In a 100 mL Erlenmeyer flask containing 50 mL sterilized Yeast Peptone Dextrose (YPD) culture, three duplicates of each clone—one of an rSb transformed with Sui p 2 hypoallergen and the other of Sb without any foreign insert gene—were inoculated. This was then placed in a rotating incubator (AvansBio) and incubated for 72 hours at 30°C at 200 rpm. A 2 mL culture was collected every 3 hours, and a spectrophotometer was used to detect the optical density of the sample at 600 nm every three hours. The growth curve pattern was then plotted using GraphPad Prism version 10 software (GraphPad Software Inc., California, USA) for easier visualization.

Dot Blot Assay. T Cell lysates from two rSb clones, Rab7 Sb for a negative control, and T5 Sb for a positive control were prepared using the YD Digestion Buffer and 5µL of R-Zymolyase (RNaseA+Zymolyase). Each clone's 10 mL culture was prepared and incubated at 30°C and 200 rpm in a rotary incubator (AvansBio) for three days before lysis. Cell lysates were directly blotted onto a nitrocellulose membrane in duplicates. For one hour at room temperature, 5% skimmed milk in 1X PBS-T was used to block the blotted membrane. Detection of recombinant hypoallergens and positive control was done using mouse monoclonal anti-polyhistidine antibodies (1000x in PBS-T) for 16 hours at 4°C with continuous gentle shaking. The blots were then treated with Biotin-conjugated anti-mouse immunoglobulin antibody 2,000X in PBS-T (Sigma-Aldrich), followed by incubation with Streptavidin-HRP Conjugate 1:3000 dilution in PBS-T for 1 hour at room temperature.

A 3,3′,5,5′-tetramethylbenzidine (TMB) substrate was used to detect positive signals, and the process was halted by washing the substrate with distilled water. In between procedures, membrane blots were washed three times with 1X PBS-T.

Results and Discussion

Hypoallergenic Sui p 2. To develop a hypoallergenic variant of the Sui p 2 allergen, a structure-based rational design approach was employed, incorporating epitope mapping, allergenicity prediction, and toxicity analysis. Structural modeling and epitope analysis were performed using SWISS-MODEL to generate a three-dimensional model of the modified Sui p 2 protein (Figure 1A) The corresponding amino acid sequence of the engineered hypoallergenic variant designed using various computational tools—is depicted alongside the model with immunologically relevant regions annotated. Discontinuous epitopes are indicated in red, linear epitopes are outlined with dashed boxes, and residues subjected to alanine substitution are highlighted in green. These substitutions were strategically introduced within IgE-binding regions to disrupt allergenic determinants while preserving the overall tertiary structure of the protein. Allergenicity prediction was also conducted using the AlgPred 2 algorithm (Figure 1B). The hypoallergenic variant exhibited a reduced machine learning (ML) score (0.44) compared to the native protein (0.52). Similarly, the hybrid score, which integrates multiple predictive features, decreased from 1.02 in the native form to 0.94 in the engineered variant. While both versions were still classified as allergens, the observed reduction in predictive scores suggests a diminished allergenic potential in the modified protein.



B

Subject	ML Score	MERCI Score	BLAST Score	Hybrid Score	Prediction
Native	0.52	0	0.5	1.02	Allergen
Hypoallergen	0.44	0	0.5	0.94	Allergen

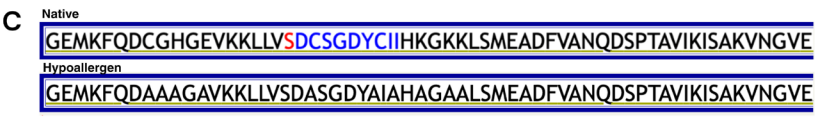


Figure 1. In Silico Analysis of Hypoallergenic Sui p 2. Structural model of the hypoallergenic Sui p 2 protein generated using SWISS-MODEL. The corresponding modified amino acid sequence is shown on the right, with discontinuous epitopes highlighted in red, linear epitopes boxed with dashed lines, and alanine-substituted residues shown in green (A). Allergenicity prediction using the AlgPred 2 algorithm (B). Toxicity prediction comparison between native and hypoallergenic sequences. Residues in red color represent the starting residue of toxic stretch and those in blue color represent the trailing residues falling in toxic stretch (C).

The observed reduction in both the machine learning (ML) score and the hybrid score for the hypoallergenic variant, relative to the native Sui p 2 protein, indicates a general decrease in predicted allergenic potential. The lower ML score reflects a reduced likelihood of the protein being classified as an allergen based on key sequence features identified by trained predictive models. In parallel, the decrease in the hybrid score which synthesizes multiple algorithmic predictions including motif patterns, sequence similarity to known allergens, and physicochemical characteristics—suggests a consistent trend toward diminished allergenicity.

In addition, a toxicity analysis was also performed using a toxin prediction algorithm to evaluate the safety profile of the protein variants (Figure 1C). A toxic stretch was identified in the native Sui p 2 sequence, with initiating residues marked in red and the corresponding toxic segment highlighted in blue. Notably, this predicted toxic region was absent in the hypoallergenic variant, indicating that the introduced mutations effectively eliminated toxicity-associated sequences. Hence, these results demonstrate that targeted mutagenesis of epitope regions in Sui p 2 leads to a hypoallergenic variant with reduced allergenicity and toxicity, while maintaining structural integrity. This highlights the potential of structure-guided protein engineering in the development of safer immunotherapeutic candidates.

hSui p 2 gene integration into Saccharomyces boulardii genome. A 666 bp insert, comprising a 267 bp alpha-factor sequence with a start codon, a 378 bp hSui p 2 open reading frame, an 18 bp six-histidine tag, and a stop codon, was cloned into a yeast-integrating plasmid, generating a 4571 bp pYIP-Derp2/1S plasmid construct (Figure 2A). The plasmid vector includes the URA3 auxotrophic selection marker, a TEF1 yeast promoter, a Kozak sequence for efficient eukaryotic translation, and an alpha-factor signal for recombinant protein secretion. It also contains a CYC1 terminator and a StuI restriction site for linearization, enabling genomic integration via homologous recombination. Additionally, the vector carries a pUC Ori for high-copy replication and an ampicillin resistance gene for selection in *E. coli*. Moreover, the plasmid was further verified by linearization using a StuI restriction enzyme and confirmed through agarose gel electrophoresis (Figure 2B). Subsequently, the plasmid construct was transformed into *E. coli* via the heat shock method using calcium chloride and was grown in LB plates. Glycerol stocks were then prepared for long-term storage at -80°C.

Subsequently, the URA3 gene in the *hSui* p 2 plasmid facilitated its linearized integration into the *Saccharomyces boulardii* (Sb) genome via homologous recombination. This integration enabled the transformation of *S. boulardii* into recombinant *S. boulardii* (rSb). To confirm transformation, rSb was selected on fresh SD-URA medium, where colony growth indicated successful homologous recombination. The presence of the URA3 gene allowed the strain to proliferate in uracil-deficient conditions. Polymerase chain reaction (PCR) further validated transformation by detecting the 464 bp *hSui* p 2 gene in two Sb transformant clones (Figure 2 C).

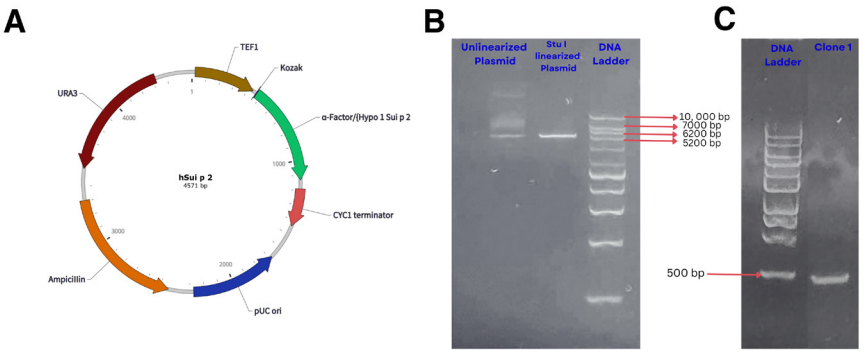


Figure 2. Structure of the plasmid vector pYIP-hSui p 2 construct (A) with a total size of 4571 bp as confirmed through StuI linearization (B). The hSui p 2 insert was PCR-amplified from the genomic DNA of one clone of transformed Sb (C) using primers that amplified a 464 bp amplicon.

Expression of the His-tagged hSui p 2. hSui p 2 was expressed as a recombinant protein with a 6xHis-tag in Sb. Dot blot immunoassay using mouse anti-His antibodies showed positive expression of the His-tagged recombinant hSui p 2 in two clones of Sb (Figure 3) with confirmed hSui p 2 gene integration into the Sb genome (Figure 2C). The specificity of the anti-His antibodies were confirmed with the detection of a His-tagged T5 RBD protein but not the Rab7-expressing Sb.

The dot blot assay confirmed the successful expression of hSui p 2 by recombinant *Saccharomyces boulardii* (rSb), reinforcing its potential as a platform for prophylactic delivery. Numerous studies have demonstrated the effectiveness of *S. boulardii* as an oral vaccine delivery system, primarily due to its probiotic nature and resilience within the gastrointestinal (GI) tract. These characteristics make *S. boulardii* a promising candidate for antigen delivery, particularly in eliciting mucosal immunity [30]. a study by Pan et al., emphasized several advantages of *S. boulardii* in antigen transport, including its ability to efficiently express and deliver recombinant proteins, stimulate both systemic and mucosal immune responses, and serve as a cost-effective alternative to conventional vaccine platforms. Furthermore, its innate resistance to stomach acidity and enzymatic degradation enhances antigen stability, making it particularly suitable for mass vaccination initiatives, especially in resource-limited regions. Additionally, *S. boulardii* has been reported to contribute to gut health and modulate immune function, further supporting its potential as a vehicle for vaccine and therapeutic protein administration.

Beyond its role in antigen expression, *S. boulardii* actively interacts with the mucosal immune system by stimulating secretory IgA production, regulating cytokine expression, and promoting immune homeostasis. These immunomodulatory properties, coupled with its ability to survive the harsh GI environment, highlight its stability and efficacy as a delivery system [31]. Moreover, most recombinant proteins expressed in *S. boulardii* are His-tagged, enabling efficient identification and verification of successful expression. The polyhistidine tag (His-tag) is widely utilized in recombinant protein purification, consisting of a sequence of six histidine residues fused to either the N- or C-terminal region of the protein.

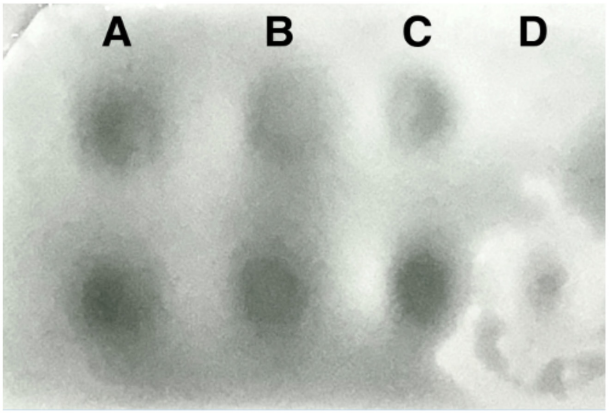


Figure 3. Dot Blot Assay identifying the six histidine-tag in rSb expressing hSui p 2. T5-expressing Sb (A), a positive control; hSui p 2-expressing Sb Clone 1 (B) hSui p 2-expressing Sb Clone 2 (C); and Rab7-expressing Sb (D), a negative control.

While the optimal positioning of the His-tag varies depending on the protein, the N-terminal His-tag is more commonly used in bacterial expression systems. Due to its short sequence, the 6×His-tag minimizes structural alterations, preserving the protein's conformation and functionality, such as in the case of this study [32].

Growth Kinetics of Recombinant S. boulardii integrated with hSui p 2. The growth kinetics of *Saccharomyces boulardii* (Sb) and recombinant *Saccharomyces boulardii* (rSb) were analyzed based on optical density (OD_{660}) measurements over a 30-hour period. Both strains exhibited a distinct lag phase during the initial hours, where minimal growth was observed as the cells adapted to the culture conditions. This adaptation period was similar for both Sb and rSb, indicating that the insertion of a recombinant gene did not significantly affect the initial phase of growth.

Following the lag phase, both strains entered the exponential phase, where rapid cell division led to a steady increase in OD_{660} values. However, Sb displayed a slightly faster growth rate compared to rSb (Figure 4). This difference suggests that the recombinant strain experienced a metabolic burden due to the additional load of expressing a foreign protein, slowing its growth progression [33]. Despite this, both strains followed a similar growth trend, with rSb maintaining a comparable but slightly delayed rate of increase in biomass.

During the stationary phase, Sb reached its peak OD_{660} of 3.036 at approximately 27 hours, while rSb attained a slightly lower peak OD_{660} of 2.966 at 30 hours. The delayed peak of rSb further supports the idea that recombinant protein expression imposes an additional energy demand, slightly prolonging its growth cycle and reducing its overall biomass yield. After reaching their respective peaks, both strains entered the decline phase, with Sb beginning to decrease in OD_{660} earlier than rSb. This earlier decline suggests that Sb exhausted its nutrients or accumulated inhibitory metabolic byproducts more quickly than rSb, which maintained its peak for a longer period before declining.

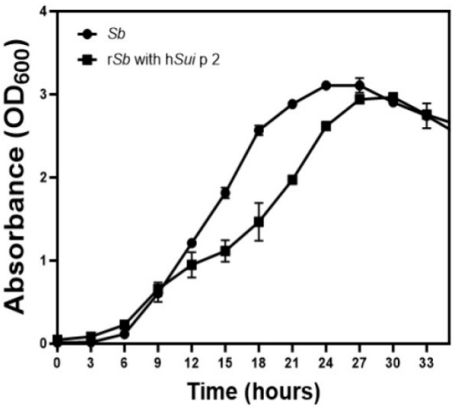


Figure 4. Growth Kinetics of Sb and rSb.

The elevated energy demands and metabolic strain associated with the high expression of recombinant proteins contribute to the slower growth of rSb. This is particularly evident in slightly reduced peak growth. However, despite the slower progression, rSb ultimately reaches a growth level comparable to that of Sb, with no significant difference in its maximum growth yield. Overall, the results highlight the impact of recombinant protein expression on yeast growth. While both strains followed similar growth patterns, rSb exhibited a slower growth rate, a delayed peak, and a slightly lower biomass yield compared to Sb.

Conclusion

This study successfully characterized, cloned, and expressed hypoallergenic Sui p 2 in *Saccharomyces boulardii*, demonstrating its potential as a novel system for allergen-specific immunotherapy (ASIT) and a prophylactic agent against house dust mite (HDM) allergy. The results confirm that recombinant *S. boulardii* can effectively express Sui p 2 while maintaining hypoallergenicity, fulfilling key preclinical requirements for vaccine development. These findings mark a significant step forward in creating safer and more effective ASIT and prophylactic solutions, paving the way for future clinical applications in allergy treatment.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Experimental research, methodology, data analysis and interpretation, original draft preparation, review and editing of the draft, C.Y.P. Conceptualization for N.P.G.R.A. Conceptualization, data analysis and interpretation, review, and editing of the draft, supervision, J.D.A.R. All authors have read and approved the final manuscript.

Institutional Review Board Statement

All work involving infectious or potentially infectious biological agents, materials, toxins, and genetically modified organisms (GMOs) was conducted in accordance with the biosafety guidelines and received clearance from the Institutional Biosafety Committee. Specifically, approval was granted by the University of Santo Tomas (UST) Institutional Biosafety Committee, with authorization confirmed by Dr. Gina Dedeles on April 7, 2025.

Informed Consent Statement

Not applicable.

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