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# Display of wasp venom allergens on the cell surface of yeast

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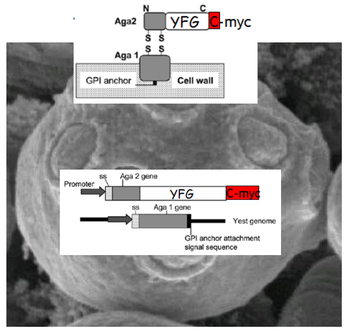
## ABSTRACT

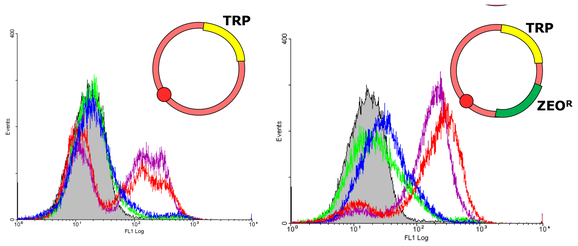
Yeast surface display is a technique, where the proteins of interest are expressed as fusions with yeast surface proteins and thus remain attached to the yeast cell wall after expression. Our purpose was to study whether allergens expressed on the cell surface of baker's yeast *Saccharomyces cerevisiae* preserve their native allergenic properties. We chose to use the major allergens from the common wasp *Vespula vulgaris* venom (phospholipase A1 PLA1, hyaluronidase HYA and antigen 5) as the model.

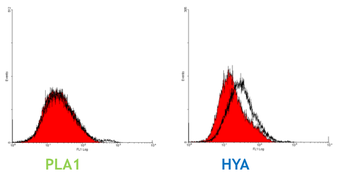
## EXPRESSION ON THE YEAST SURFACE

The genes encoding phospholipase A1 (*ves v1)*, hyaluronidase (*ves v2*) and antigen 5 (*ves v5*) were obtained by RT-PCR from *V. vulgaris* venom sac RNA. The proteins were expressed on the surface as fusions with a-agglutinin complex protein AGA2 [Chao et al, 2006]. In order to express PLA1 and HYA, we modified the surface display expression vector to include antibiotic resistance casette so that constant selective pressure could be applied. HYA and antigen 5 expression was confirmed by fluorescent cytometry (FACS) analysis of cells stained with FITC-conjugated anti-C-myc antibody (green fluorescence FL1). The expression of PLA1 was too low to be detected by FACS.

On the figure expression of reference protein CD20, PLA1, HYA, antigen 5, non-expressing cells. Left – tryptophan selection, right – tryptophan and zeocin selection.
Below: PLA1 and HYA-expressing cells have high autofluorescence. Non-labeled (red background), labeled (black).



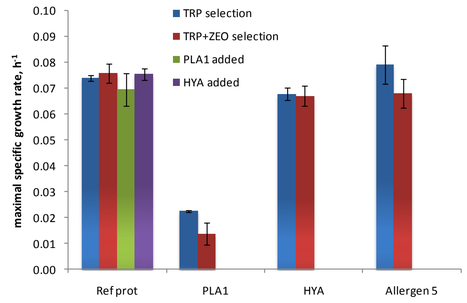




## ENZYMATIC ACTIVITY AND YEAST GROWTH INHIBITION

PLA1 and HYA expressed on the surface retained their enzymatic activites. PLA1 expression severely inhibited growth of the yeast cells.

| Plasmid selection | Activity, units/$10^9$ cells | |
|---|---|---|
| | Phospholipase A1 | Hyaluronidase |
| Tryptophan | 0.08 | 31 |
| Tryptophan and zeocin | 2.6 | 90 |




## REFERENCES

Chao G, Lau WL, Hackel BJ, Sazinsky SL, Lippow SM, and Wittrup KD. 2006. *" Isolating and engineering human antibodies using yeast surface display."* Nat. Protocols 1(2):755-768

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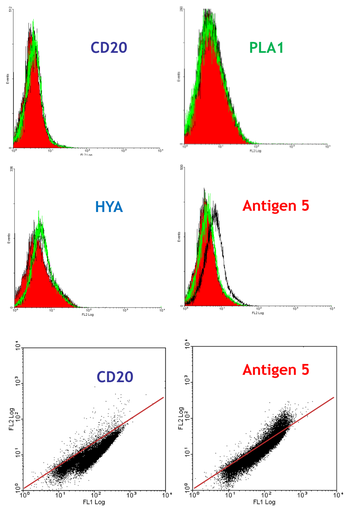
## BINDING OF HUMAN IgE

The allergen binding to human IgEs was also studied by FACS. The binding of IgE was detected by biotinylated anti-IgE antibody and streptavidin-PE (red fluorescence FL2). HYA bound IgE antibodies from hypersensitive patients serum and from the control non-hypersensitised serum pool. The antigen 5 bound IgE antibodies from hypersensitised sera pool and did not react with control sera.

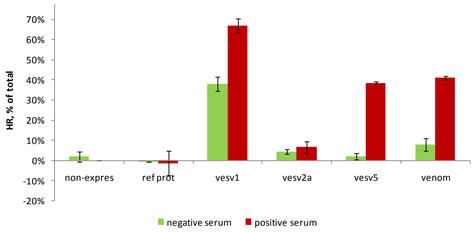
On the figure: non-labeled cells (red background), cells incubated with control sera pool (green) and cells incubated with positive sera pool (black).

Double staining with anti-C-myc antibody (FITC) and human IgEs shows that expression of full-length allergen correlates with IgE binding.




## HISTAMINE RELEASE

The allergen-expressing cells were tested for their ability to release histamine from periferal whole blood basophils charged with hypersensitive and control serum IgEs [www.reflab.dk]. All the allergen-expressing cells caused histamine release though to different extents. PLA1-expressing cells also caused some response from basophils charged with control sera IgEs.

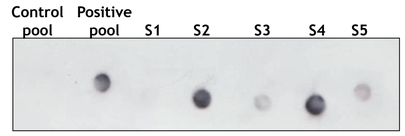



## SERUM PROFILING USING ANTIGEN 5-EXPRESSING YEAST

We tested variation of antigen 5 binding in five hypersensitive patients sera by FACS and HR tests using antigen 5-expressing yeast cells. The variation of antigen 5 binding to different sera was also tested by immunoblot with recombinant antigen 5.

One out of five sera did not react with Ves v5 as confirmed by all analyses. Other four sera reacted with Ves v5 with different stregth. Interestingly, while HR and dot blot results had similar distribution of signal stregth, the FACS data did not correlate with those results. It can be connected to the fact that in FACS analysis there was an excess of antibodies while in other analyses there was an excess of antigen.

| Serum | FACS, mean ratio | HR, % total |
|---|---|---|
| Control pool | 0.9 | 0.7 |
| Positive pool | 1.4 | 9.4 |
| Serum 1 | 0.8 | 1.2 |
| Serum 2 | 1.2 | 6.9 |
| Serum 3 | 3.9 | 2.4 |
| Serum 4 | 1.6 | 13.0 |
| Serum 5 | 2.6 | 2.5 |




## CONCLUSIONS AND FUTURE WORK

In conclusion, the allergens expressed on the surface of yeast retained their IgE binding capabilities, caused histamine release and remained enzymatically active (where applicable). In perspective the yeast surface display can be used for allergen discovery from cDNA libraries and possibly for sublingual specific immunotherapy (SLIT) as the cells can be produced in large amounts at a low price.