Development of antibodies for cyanobacterial neurotoxin: anatoxin-a

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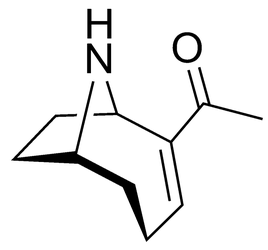
MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Introduction

Harmful algal blooms are an occurence seen worldwide in our waters. Cyanobacteria are often present in these blooms where they produce several different harmful toxins, one of the most common being anatoxin-a. It is a very small molecule with a molecular mass of around 165 Da and it is classified as a neurotoxin. Some negative implications of this toxin include toxicity to animals and livestock, irritation and illness in humans if ingested and impacting water quality.

Harmful algal bloom (CDC, 2023)



Anatoxin-a structure

Purpose of this research

While testing this toxin is vital for enviromental and safety reasons, current immunoassay based testing methods are limited due to lack of availability of the antibody.

The aim of this thesis was to develop antibodies against anatoxin-a. This could then pave the way to develop a simple and cost-effective test against anatoxin-a.

Methods

First, three Fab phage display libraries, which vary by having a different source of variable light chain (VL) were panned three times against a synthetic biotin-anatoxin complex (Fig. 1&2). The Fab DNA from the phage stocks that showed good enrichment were cloned into *E.coli* to produce soluble Fab (Fig. 3) and screened by a competitive immunoassay (Fig. 4).

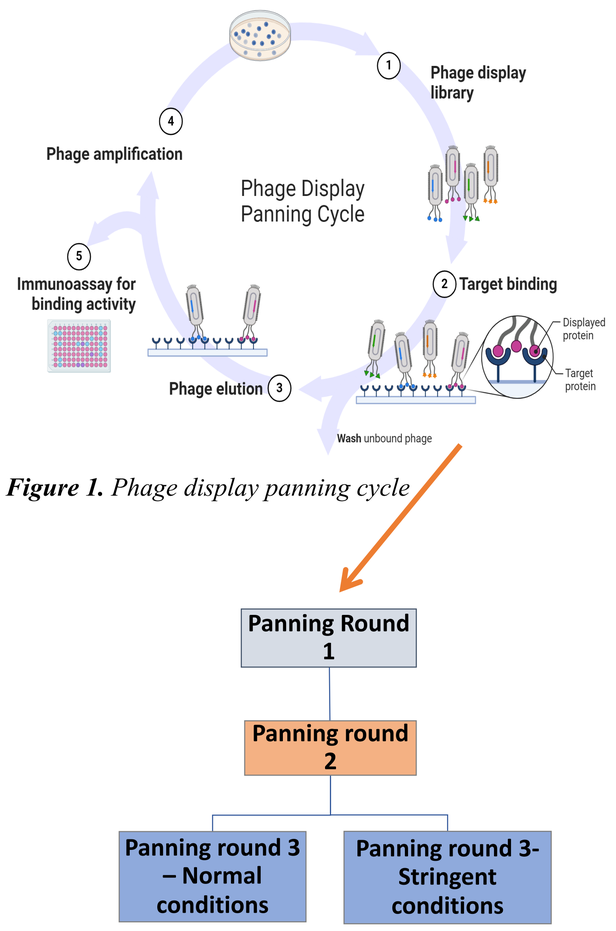


Figure 1. *Phage display panning cycle*

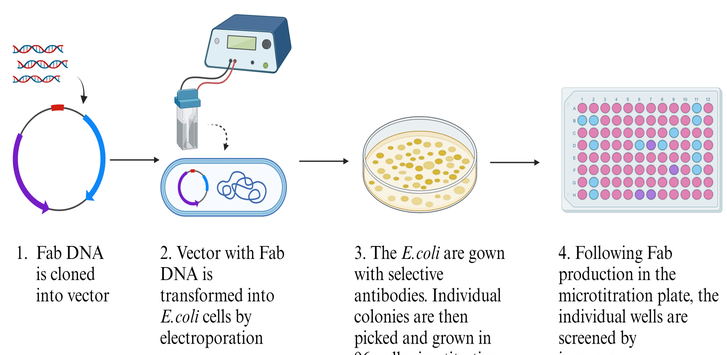


Figure 3. *Cloning and production of soluble Fab*

Figure 2. *Phage display panning against biotinylated anatoxin*

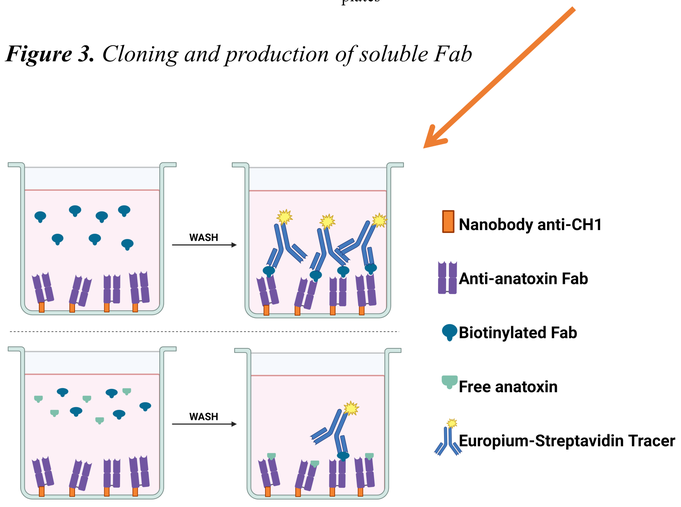


Figure 4. *Screening competitive immunoassay*

As no good binders were found in the initial stage, the panning was repeated this time using HRP-anatoxin conjugate as the target protein (Fig. 5). Following cloning and production of soluble Fab (Fig. 3), the clones were screened with an immunoassay against both biotin- and HRP-anatoxin (Fig. 6):

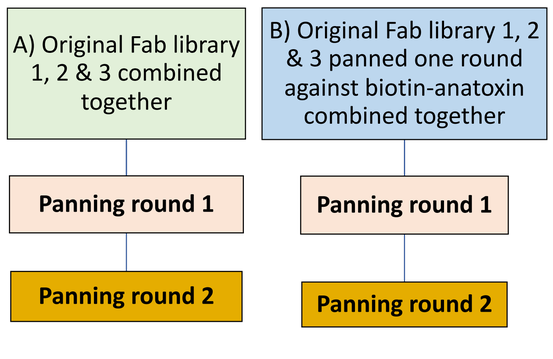


Figure 5. *Phage display panning against HRP-anatoxin*

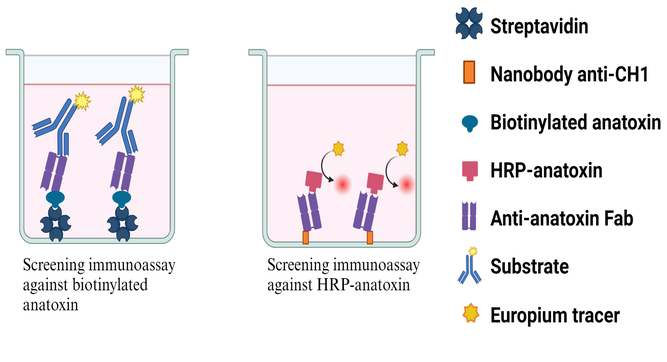


Figure 6. *Screening with biotin- and free-anatoxin*

Results

From the possible anti-anatoxin Fabs obtained from panning against biotin-anatoxin complex, 16 clones out of 1330 clones screened were showing significant binding during the competitive assay (Fig.7).

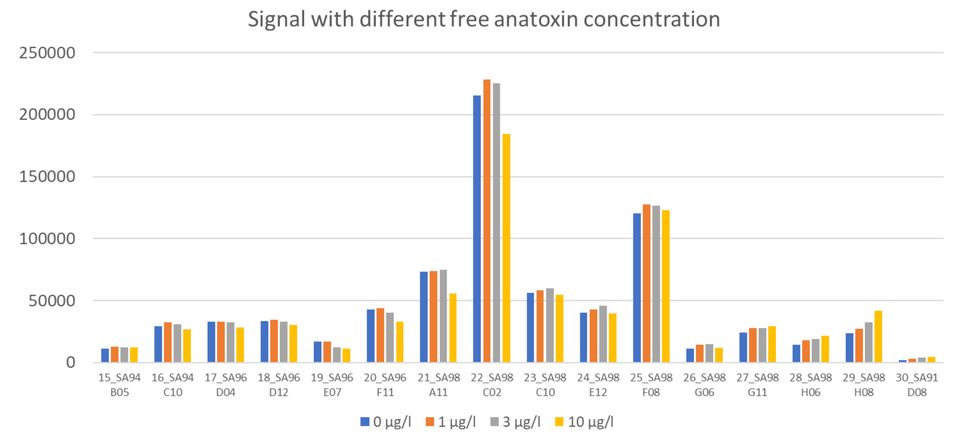


Figure 7. *Signals obtained during a competitive immunoassay with free- and biotinylated anatoxin*

The 16 clones were then tested to see how well they bind to HRP-anatoxin, however, they did not bind.

When the panning was repeated against HRP-anatoxin, the Fabs produced were screened to check binding to biotin-anatoxin and HRP-atx. A total of 752 clones were screened and the best 30 clones were selected. Fig. 8 shows the signal/background ratio (how well Fab is binding to specific target) of the 30 clones from both immunoassays.

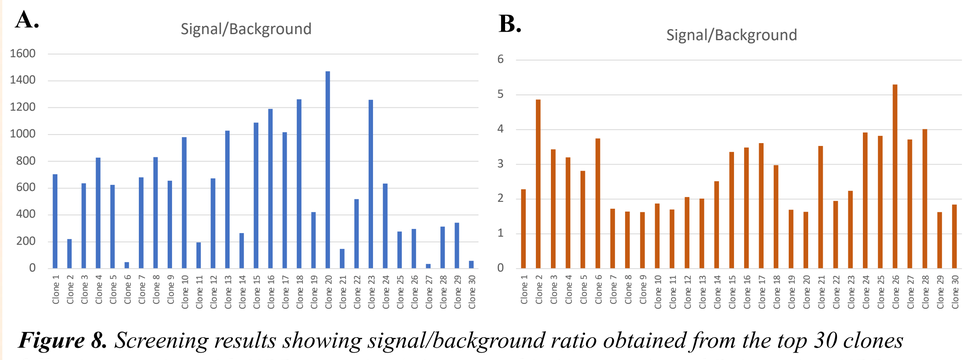


Figure 8. *Screening results showing signal/background ratio obtained from the top 30 clones following panning with HRP-anatoxin. A. is against biotin-anatoxin and B. is against HRP-anatoxin.*

Conclusion

- From the first panning experiment done against biotinylated anatoxin, no good binders were found.
- However, the initial panning against biotin-anatoxin followed by subsequent panning with HRP-anatoxin still lead to finding 30 Fab binders to both biotin-anatoxin and HRP-anatoxin.
- We hypothesize that this means they could bind to free anatoxin. However as the next step further analysis of these 30 clones needs be carried out to characterize them and confirm that they bind to free anatoxin

References

- CDC (2023) Harmful Algal Bloom (HAB)-Associated Illness. Available at: https://www.cd c.gov/habs /index.html
- Climate Central (2022) Toxic Algae Blooms in a Changing Climate. Available at: https://www.climatecentral.org/climate-matters/harmful-algal-blooms
- Chorus, I. and Welker, M. (2022) Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and Management. Boca Raton: CRC Press
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