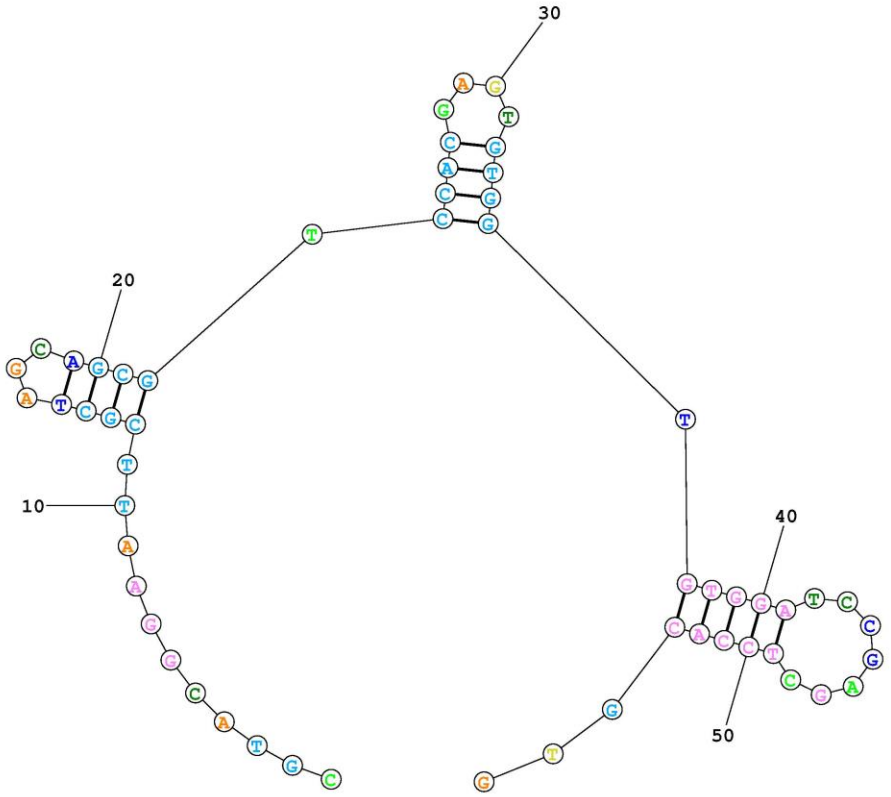
Development of an enzyme-linked aptamer-sorbent assay (ELASA) for the detection of Tebuconazole in wood

Kathrin Kusstatscher BSc.



Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50% > Probability

ENERGY = -14.4 09/03/17 04:10:12

- fast, simple, cost effective
- aptamers:
  - ssDNA
  - high affinity to target
  - artificial synthesis, in vitro selection, stability
  - used as molecular recognition elements
  - high number of possible targets
  - high number of possible detection methods

https://rna.urmc.rochester.edu/RNAstructureWeb/Servers/Predict1/ResultsPages/20170903.041012-5e81eb30/Results.html

Nguyen, V.-T., Kwon, Y.S., Kim, J.H., Gu, M.B. (2014). Multiple GO-SELEX for efficient screening of flexible aptamers. *Chemical Communications*, **50** (72), 10513-10516.

DEVELOPMENT OF AN ENZYME-LINKED APTAMER-SORBENT ASSAY (ELASA) FOR THE DETECTION OF TEBUCONAZOLE IN WOOD

Kathrin Kusstatscher*[1,2], Margit Cichna-Markl[2], Sabrina Niedermayer[1], Notburga Pfabigan[1], Martin Weigl[1], Roland Gründlinger[1]

[1] Holzforschung Austria, Franz Grill-Straße 7, 1030 Wien; www.holzforschung.at, Tel +43-1-798 26 23-0
[2] Department of Analytical Chemistry, University of Vienna, Währinger Strasse 38, 1090 Wien; www.anchem.univie.ac.at
*corresponding author, Tel +43-1/7982623-834; k.kusstatscher@holzforschung.at

INTRODUCTION

The aim of this study was to develop a fast, simple and cost effective detection method for the fungicide Tebuconazole based on aptamers. Aptamers are single stranded nucleic acids that bind to a target molecule with high specificity and can be selected in vitro by the SELEX process. Different studies report that the affinity of aptamers to their target can be compared with the affinity of antibodies to their antigen. Aptamers can be applied as recognition elements in a broad range of detection methods. The method applied in this work was a competitive enzyme-linked aptamer-sorbent assay (ELASA), first described by Gu et al., 2016. The aptamer used has been reported as specific for Tebuconazole by Nguyen et al., 2014. The affinity of the aptamer to Tebuconazole was assessed by experiments using graphene oxide (GO) and NanoDrop measurements.

METHOD

The ELASA (shown in Figure 1) included the following steps:

1. Coating of the microtiter plate with avidin or streptavidin
2. Blocking of remaining binding sites to avoid unspecific binding
3. Immobilization of the aptamer (sequence shown in Tab. 1) via avidin/streptavidin-biotin binding
4. Hybridization of the biotinylated complementary sequence (CS) to the aptamer
5. Addition of Tebuconazole and competition with the CS for aptamer binding
6. Binding of enzyme labelled avidin/streptavidin to the CS
7. Addition of tetramethylbenzidine (TMB) substrate and H_2O_2 for enzyme reaction
8. Stopping of the enzyme reaction using 0.5 M H_2SO_4
9. Measurement of the absorbance at 450 nm

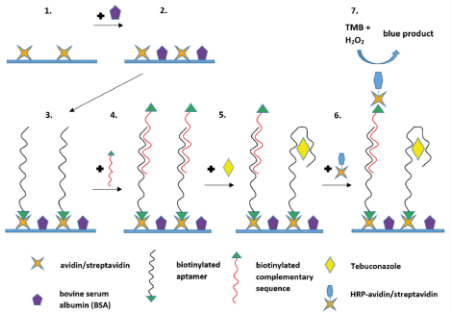


Fig. 1: Schematic illustration of the principle of the ELASA. (TMB: tetramethylbenzidine, HRP: horseradish peroxidase).

The parameters varied to obtain a suitable calibration curve, were the coating reagent (avidin and streptavidin) and concentration, blocking reagent (BSA and casein) and concentration, aptamer pre-treatment (denaturation and cooling to room temperature or on ice; no treatment) and concentration, different CS (Table 2) and their concentrations and enzyme labelled reagent (HRP-avidin/streptavidin) and concentration.

NAME	SEQUENCE
APTAMER T1	5'Biotin-TEG-CGT ACG GAA TTC GCT AGC AGC GTC CAC GAG TGT GGT GTG GAT CCG AGC TCC ACG TG-3'

Tab. 1: Sequence of the aptamer specific to Tebuconazole.

RESULTS

The following figures show some results obtained from ELASA and affinity determination of the aptamer to Tebuconazole.

NAME	SEQUENCE
FCS	5'Biotin-TEG-AAAACTCGGATCCACACCACACT
sFCS	5'Biotin-TEG-AAAACTCGGATCCAC
CSM2	5'Biotin-TEG-AAAACTCGGATAAACACCACACT
CSM3	5'Biotin-TEG-AAAACTCGGATAATCACCACACT
CSM4	5'Biotin-TEG-AAAACTCGGATAATAACCACACT
CSM5	5'Biotin-TEG-AAAACTCGGAAAATAACCACACT

Tab. 2: Differently modified CS used for assay optimization.

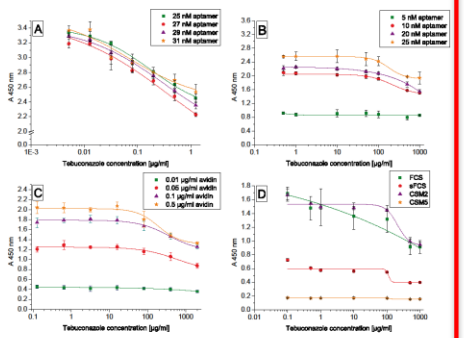


Fig. 2: Influence of different optimization steps on the shape of calibration curve. A: avidin concentration, B: aptamer concentration, C: aptamer concentration at higher Tebuconazole concentration, D: different CS.

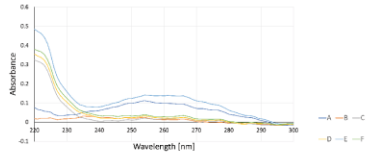


Fig. 3: Absorption spectra obtained from NanoDrop measurement. A: aptamer, B: aptamer + GO, C: Tebuconazole, D: Tebuconazole + GO, E: aptamer + Tebuconazole, F: aptamer + Tebuconazole + GO.

CONCLUSION

Although promising results were received in some experiments, a repeatable method could not be developed. The verification of the affinity of the aptamer to Tebuconazole showed, that the aptamer has no or low affinity to its target. However, since aptamers are reported as promising recognition elements, the selection of a new aptamer for Tebuconazole or other target molecules will be our further prospect.

REFERENCES

Nguyen VT, Kwon YS, Kim JH, Gu MB (2014): Multiple GO-SELEX for efficient screening of flexible aptamers. Chemical Communications 50: 10513-10516.

Gu H, Duan N, Wu S, Hao L, Xia Y, Ma X, Wang Z (2016): Graphene oxide-assisted non-immobilized SELEX of okdaic acid aptamer and the analytical application of aptasensor. Scientific Reports 6: 21665.

COST FP 1303 - FINAL CONFERENCE
Building with Bio-Based Materials:
Best practice and performance specification
6th – 7th September, 2017 - Zagreb, Croatia

HOLZ FORSCHUNG AUSTRIA

Kathrin Kusstatscher BSc.
k.kusstatscher@holzforschung.at
Tel. +43/1/798 26 23-834

www.holzforschung.at