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Towards a 2025 Global Beer Market: The Use of Aptamers in Biosensors to Detect *Acetobacter Aceti* in Beer - A Biotechnological Approach to Improve Food Hygiene and Safety in Beer Developing Markets and Beyond

Goshen David Miteu

School of Biosciences, Biotechnology, University of Nottingham, England, United Kingdom
School of Medicine and Health Sciences, Department of Biomedical Science, University of North Dakota, United States.
*Corresponding author email: goshendavids@gmail.com ; goshen.miteu@und.edu

Abstract

Aptamers were studied to see if they could be designed as a rapid detector for viable spoilage organisms in beer. SELEX was used to obtain aptamers from random libraries of oligonucleotides that could bind to *A. aceti*. 13 SELEX rounds were performed to get specific aptamers, whole and counter cell-SELEX was employed. Aptamers' binding ability to *A. aceti* was tested by flow cytometry and confirmed to bind by synthesizing them with fluorescent labels (5'FAM). ANOVA and a one-sample test revealed significant difference in the binding ability of selected aptamers ($P < 0.05$). A12 and A10 showed the highest fluorescent intensity (180 and 150) for binding ability. Flow cytometry test for the binding specificity of aptamers and their truncated sequence in comparison with other organisms revealed selected aptamers to be specific to *A. aceti* cells having the highest mean fluorescence intensity values of 120 ± 3.8 (A12), 119 ± 5.2 (TA12), 110 ± 4.5 (A10) and 111 ± 6.2 (TA10). The best predicted folding structure of selected aptamers from mFold was determined from the Gibbs free energy (A10: $\Delta G = -4.40$, TA10: $\Delta G = 0.07$, A12: $\Delta G = -4.96$, TA12: $\Delta G = 0.43$). Sigmaplot showed no dose-dependent relationship for detection in candidate aptamers. A12 had a low KD value of 11.22 ± 1.32 nM which further validated it as the best aptamer for detecting *A. aceti* in beer.

Keywords: *Aptamers, Aptasensors and biosensors, Oligonucleotides, Beer, Spoilage organisms, Acetobacter aceti in beer, Food hygiene*

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1. Introduction

One of the most consumed and oldest alcoholic beverages in the world is beer. It is also the third most popular drink after tea and water. As the global beer market increases with a future projection of £688 billion by 2025 (Statista, 2022), there is a need to reduce the amount of spoilage organisms and contamination for sustainability in the market. Continents like Africa and South America are significant contributors to this goal. Thus, they have received attention on personal hygiene in craftsmanship, particularly brewing and distribution.

Acetobacter aceti (*A. aceti*) have been implicated in beer quality. It causes spoilage in beer upon acetification from ethanol in the presence of little oxygen which suggests that if oxygen gains entrance through wooden barrels during beer storage can facilitate spoilage by *A. aceti*. *A. aceti* is a gram-negative bacterium that is ubiquitous. It is naturally benign and nonpathogenic to humans. However, it can become a foe, as seen in beer spoilage. Acetification of beer causes the development of bacteria in beer or wort (Bouchez A and De Vuyst, 2022), and this contamination can cause diminished flavour, colour and taste in beer (Trček *et al.*, 2015; Fernandez *et al.*, 2010). Furthermore, this bacteria can cause biofilm formation which can lead to pathogenic infestation (Toriya *et al.*, 2010; Yetiman, 2015).

Food Standard Agency has reported that contamination must be avoided for consumer goods to remain in markets. Detecting organisms like *A. aceti*, especially in developing countries is critical for the business to thrive internationally. Only smart detection technologies like apta-sensors can prompt relevant stakeholders to make timely decisions about beer contaminations from spoilage organisms (Lazcka *et al.*, 2007).

Conventional methods for bacterial detection like culturing could be exhaustive and time consuming which underscores the need for better detection techniques that are rapid, qualitative, and cheap. This birthed the efficient application of aptamer-based biosensors, which have been reported to be effective in detecting micro-organisms in food (Amaya-González *et al.*, 2013; Soundy and Day, 2017). An example is an electrochemical sensor that was developed to detect Mycobacterium tuberculosis (Zhang *et al.*, 2019).

Aptamers are designed by SELEX (Systematic evolution of ligands by exponential enrichment). It encompasses screening a random library of oligonucleotide by in vitro selection with

target cells and separation, amplification and purification of products (Liu and Zhang 2015; Ohuchi, 2012). This novel method to detect spoilage microorganisms utilizes short nucleotide sequence (aptamers) to bind to molecules of interest. The technique was adapted and first used by Tuerk and Gold (1990) to discover the first RNA aptamer, and Ellington and Szostak's (1990) studies on RNA aptamer binding to organic dyes.

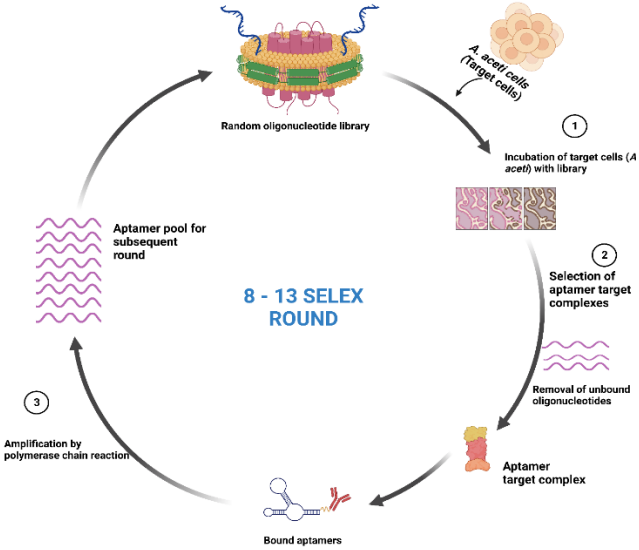

Figure 1: Schematic illustration of SELEX technique in DNA aptamers design. 10^{-4} – 10^{-5} oligonucleotide with conserved primer binding region is the initial aptamer pool. A target molecule (*A. aceti* cells) is added to allow aptamer to bind in this pool. In the absence of targets, aptamers are unstructured and will only conform and bind upon their target recognition. The target-aptamer complex is isolated, and PCR amplifies the aptamers with the most binding affinity. The step is repeated until the best aptamer is found. The aptamers from the SELEX selection are full-length sequences containing the conserved primer binding sequences (Created with BioRender.com).

Aptamers have shown great potential in pivoting one health across diverse industries because of their unique characteristics. From diagnostics to therapeutics production (Figure 2), aptamer-based tools have become an asset and a game changer. Bio-trends suggest that the potential of aptamers are limitless and yet to be fully harnessed (Niederlender *et al.*, 2021; Hong and Sooter, 2015; Yu *et al.*, 2021).

Aptamers are very selective and specific to their targets (Ohuchi, 2012). They can distinguish between proteins that are 96% similar, thus reducing the errors of false positivity. They are made by chemical synthesis from ssDNA or RNA sequences shorter than 50 nucleotides. Aptamers can take different structural motifs (Bing *et al.*, 2017) with varying sequences between lengths of 25 to 100 bases. Aptamers are produced with great accuracy, stability, and high reproducibility (Jayasena *et al.*, 1999). Breaker, 1997 and Wu *et al*. (2017) reported that DNA aptamers are preferred because of their stability as opposed to RNA aptamers, which can form complex structures.

Aptamers are now a better choice than antibodies in detection assays because they have higher binding affinity to their targets and lower dissociation constant than antibodies (Hamula *et al.*, 2006). In addition, once an aptamer is selected against a target, accurate reproducibility and purity can be guaranteed during commercial synthesis. Aptamers can be engineered and modified at their 3′ or 5′ end position to improve their features and characteristics (Ni *et al.*, 2017). Because aptamers are expected to fold in a complex three-dimensional shape, much attention has been given to how they bind to their target (Zhou *et al.*, 2017). The dissociation constant (Kd) correlates with its binding efficiency during aptamer-target interaction. A lower Kd value means a higher affinity and vice versa. A specific aptamer should bind to at least 50% of the target molecule; they can discriminate structurally similar ligands to bind to their specific targets (Ellington *et al.*, 1990).

This research used high-throughput sequencing technology and bioassays to detect *A. aceti* cells (Figure 3) for the potential design as an aptasensor (Figures 1 and 2). More importantly, this study will contribute to scaling up the beer market in developing countries such that all stakeholders involved in beer production, distribution and regulation are conscious and aware of the product's condition since it is important to maintain quality for the brewing industry to thrive in the global space while factoring in economic challenges, resources, and time.

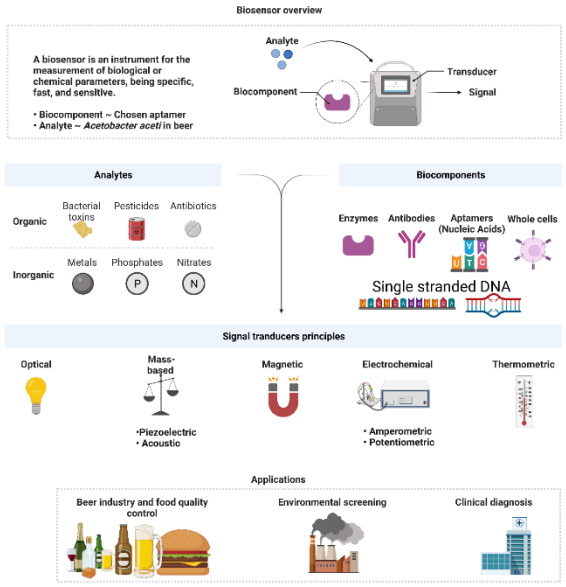

Figure 2: Biosensor overview, modelling, classification, and application. The core recognition element in this design will be the selected aptamer (Created with BioRender.com).

A potential aptamer-based biosensor that would be developed will have a biorecognition site, signal converter and a display unit for observation and analysis (Figure 2). The immobilized aptamer sequence is what will catalyze biological recognition of the analyte upon biochemical reactions, this will be the design for this aptasensor (Hianik *et al.*, 2018).

2. Materials and Methods

The method used by Liu *et al*. (2020) was adapted in this research.

Aptamers library, primers, and selection

DNA library of oligonucleotides was produced, and labelled aptamers were obtained and analyzed with an oligoanalyzer as described by UNAFold. The DNA library generated consisted of 25 nucleotides. For amplification, forward and backward

primers were created to flank both ends of the sequence culminating into a 66-measured sequence.

Forward primer: 5' -AGTATACGTATTACCTGCAGC
Backward primer: GCAAGATCTCCGAGATATCG-3'
5'-AGTATACGTATTACCTGCAGC-N25-GCAAGATCTCCGAGATATCG-3'

The selection of aptamers was conducted by methods described by Hamula *et al*. (2008). For every round of selection, fresh overnight cultures of bacteria cells were used, and the cell suspension was washed by centrifugation and resuspended with a binding buffer. DNA was denatured to ssDNA, cooled on ice for 10 minutes and mixed with cell suspension, binding buffer and BSA (to reduce nonspecific binding).

SELEX

Thirteen rounds of SELEX was done (Figure 1), and counter-selection (Figure 2) was performed after the 8th selection. Positive and negative selection (Counter and whole-cell SELEX) was done. Bound aptamers from the negative selection were discarded, while the unbound aptamers were taken for positive selection. At the positive selection, unbound aptamers were discarded while bound aptamers were retained and sequenced.

Elimination of aptamers that bind to bacteria that were not of interest was done by counter-selection after 13 rounds. The same protocol was also adopted for selecting specific aptamers, just that in this case, unbound DNA used as a new pool of aptamers after collection.

Binding ability by fluorimetry confirmation (Binding of A1 - 15 aptamers to *A aceti*)

Fluorimetry detection was used to check if aptamers can successfully bind to the pathogen of interest. Aptamers in the pool were amplified with primers by labelling with fluorescence (FAM) at their 5' region which was then visualized by signals as they bind. PCR was done and spin columns were used to purify the products.

A pool of randomly selected fluorescently labelled single stranded DNA molecules was utilized as the control and background to account for nonspecific binding. Incubation of 4 x 108 *A. aceti* cells with 300 nM aptamer generated from the ssDNA pool and random ssDNA pool was done for half an hour after which the cells were washed and resuspended in binding buffer for flow cytometric analysis.

Fluorescence of the FAM-labelled aptamers were measured with a fluorescence plate reader at a sensitivity of 85. As Liu *et al*. (2020) reported, threshold for fluorescence intensity was set to accommodate binding of more than two aptamer to *A. aceti*, this is considered as the maximum and was incubated with other cells to see if they will bind.

Mean fluorescent intensity were analyzed and was used to evaluate oligonucleotides with higher binding affinity % of fluorescent cells. ANOVA was used to analyze and visualize the fluorescence values.

Selected aptamers for *A aceti* cells

The binding of the fluorescent-labelled aptamers A1, A2, A3, A4, A5, A6, A7, A8, A9, A10, A11, A12, A13, A14, A15.

Binding specificity *A. aceti* aptamers

The sequences for A10 and A12 aptamer were labelled with fluorescence alongside their truncated sequences; TA10 and TA12 to evaluate their binding specificity. 20 µmol of fluorescently labelled A10, A12, TA10 and TA12 were incubated with strains of *A. aceti*, *S. cerevisiae* and *L. brevis*. Washing was done after incubation and the fluorescence was read with a plate reader to detect mean relative fluorescent intensity.

Binding affinity of *A. aceti* aptamers

Different concentrations of aptamers from 0-300nM were incubated with a predetermined number of *A. aceti* cells (108 CFU ml-1). The values for dissociation constants (KD) were calculated from the binding curves. For this purpose, the equation $y = Bmax\ X\ /(KD + x)$ was used in the sigmaplot 12.5 software.

Structural assessments of aptamers

The secondary structures for A12, A10, TA12 and TA10 were predicted and generated in silico with DNA mFold in silico (unafold.org).

3. Results

Aptamers selection

Figure 3 presents the selection of aptamers. Negative selection was done first, where aptamers were selected against targets in their native confirmation (it can detect and bind to targets if bacteria has some other shape or morphology in the spoilage environment). In the negative selection, cells that were not of interest (non-A. aceti cells) were first selected; a random whole cell was used other than our bacteria of interest to bind to aptamers and was eluted from the library. The unbound aptamers were retained for positive selection, i.e. using A. aceti from beer spoilage in their native conformation, aptamers that bind to these cells are chosen for amplification while the unbound aptamers are eluted (this step was repeated 8 – 14 times). It is an exponential process that generates more aptamers from the clusters. Note: After the eighth round, the negative selection was initiated again in the cycle. Negative and positive selection was used to remove the false positive binding sequence before cloning and sequencing of selected aptamers.

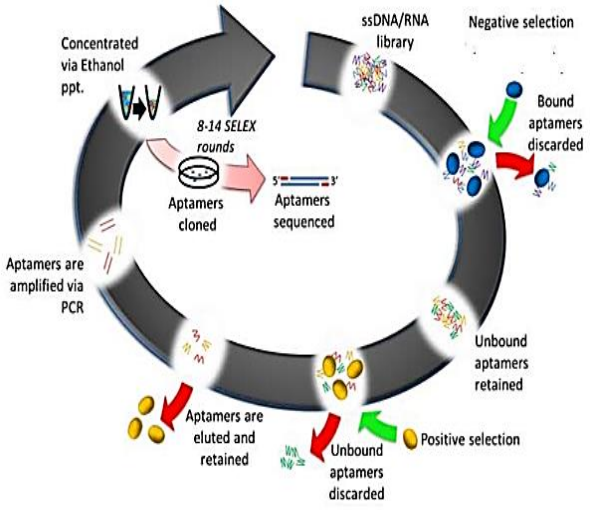

Figure 3: Selection of aptamers with SELEX.

Binding of selected aptamers to *A. aceti*

Table 1 presents the mean fluorescent intensity and binding ability of selected aptamers. A12 recorded 85% of fluorescent cells with a mean fluorescent intensity of 180.

Table 1: Mean fluorescent intensity and binding ability of selected aptamers

Aptamer	% of fluorescent cells	Mean fluorescent intensity
A1	62	50
A2	63	48
A3	80	80
A4	78	102
A5	68	50
A6	63	48
A7	81	90
A8	76	70
A10	86	150
A11	80	100
A12	85	180
A13	74	65
A14	67	50
A15	60	45

Table 2 shows the mean fluorescent intensity (RFU) of candidate aptamers. Results revealed the percentage of fluorescent cells showing a high significant mean difference of 73.071 from the test value ($t = 30.265$, $df = 13$, $p < 0.001$ for both one-sided and two-sided tests), with a 95% confidence interval ranging from 67.86 to 78.29.

Table 2: The mean fluorescent intensity (RFU) of candidate aptamers

One-Sample Test

Test Value = 0

	t	df	Significance One-Sided p	Significance Two-Sided p	Mean Difference	95% Confidence Interval of the Difference Lower	95% Confidence Interval of the Difference Upper
% of fluorescent cells	30.265	13	<.001	<.001	73.071	67.86	78.29
Mean fluorescent	7.299	13	<.001	<.001	80.571	56.72	104.42

Table 3 validates the binding ability. The analysis revealed significant differences between groups, with a sum of squares of 21977.429, 11 degrees of freedom, and an F-statistic of 19.979, corresponding to a significance level of .049.

Table 3: Validation of the binding ability.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21977.429	11	1997.948	19.979	.049
Within Groups	200.000	2	100.000		
Total	22177.429	13			

Binding specificity

Table 4 presents a comparative mean relative fluorescent intensity (RFU) values for different species upon treatment with aptamers A10 and A12, as well as their counterparts TA10 and TA12. The data reveal that *A. aceti* exhibited the highest fluorescence intensity with a mean RFU value of 110 ± 4.5 for A10 and 120 ± 3.8 for A12, indicating a higher binding affinity compared to *S. cerevisiae* and *L. brevis*.

Table 4: Mean relative fluorescent intensity (RFU) of candidate aptamers.

Species	Mean relative fluorescent intensity (RFU)			
	A10	A12	TA10	TA12
A. aceti	110 ± 4.5	120 ± 3.8	111 ± 6.2	119 ± 5.2
S. cerevisiae	19 ± 2.6	10.6 ± 2.5	20 ± 2.9	11 ± 3.6
L. brevis	13.5 ± 3.8	15.8 ± 0.9	13 ± 4.8	15 ± 1.8

Binding affinity

The binding affinity of aptamer are presented in figures 4 and 5. Result showed that after 100 nM concentration, an observed saturation was observed for A10 and A12, suggesting that increasing the concentration further does not lead to an increase in the measured response (fluorescent cells).

Figure 4 shows the binding affinity for the A10 aptamer. At the highest concentration measured, 300 µM, the A10 aptamer achieves a fluorescence intensity of approximately 80%, indicating the upper limit of its binding capacity under the conditions tested.

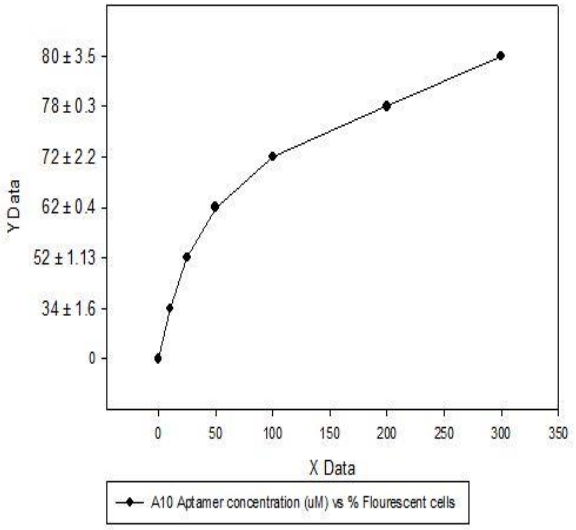

Figure 4: Binding of aptamer A10 and fluorescent cells

Figure 5 shows the binding profile of the A12 aptamer, as indicated by the percentage of fluorescent cells across varying aptamer concentrations. The result shows a fluorescence intensity mean of 78% at a concentration of 300 µM.

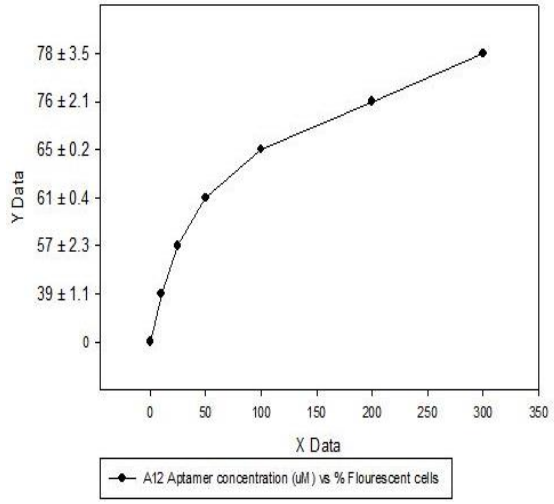

Figure 5: Binding of aptamer A12 and fluorescent cells

The optimal secondary structures for A10 aptamer, TA10 aptamer, A12 aptamer and TA12 aptamers are presented in Figures 6, 7, 8 and 9 respectively.

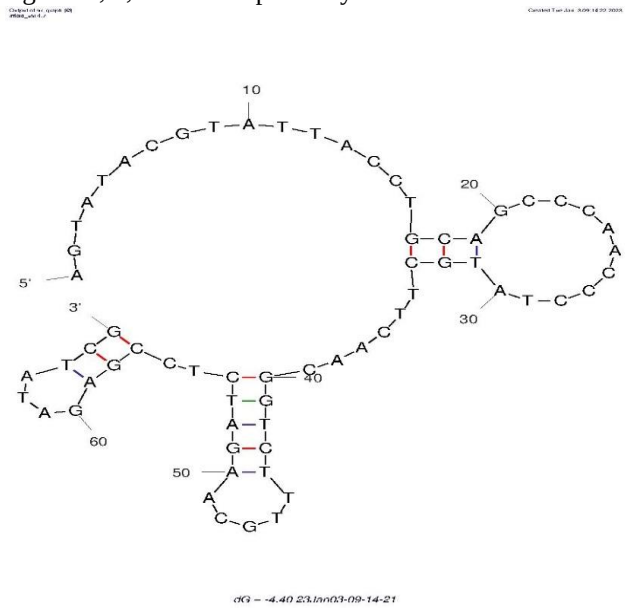

Figure 6: Representation of the optimal secondary structure for the A10 aptamer, as predicted by mFold algorithms. The best folding sequence for A10. Sequence length = 66. 17 A's, 19 C's, 12 G's, 18 U/T's, and 0 N's. ΔG = - 4.40

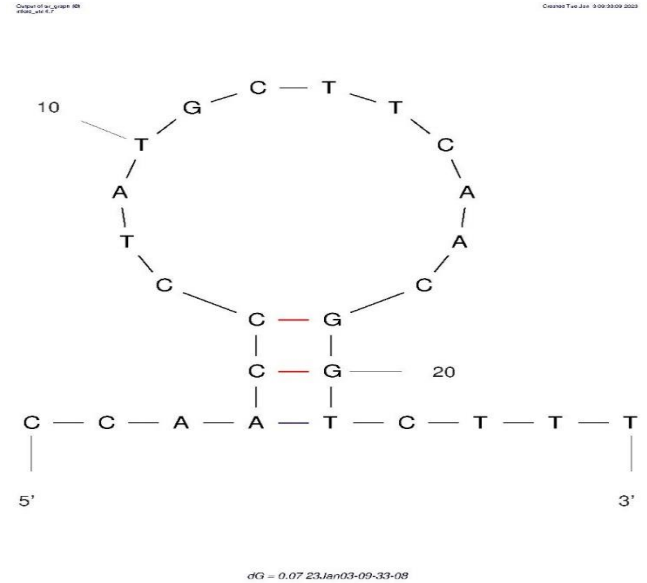

Figure 7: Visual representation of the optimal secondary structure for the TA10 aptamer, as predicted by mFold algorithms. Sequence length = 25. 5 A's, 9 C's, 3 G's, 8 U/T's, and 0 N's. ΔG = 0.07

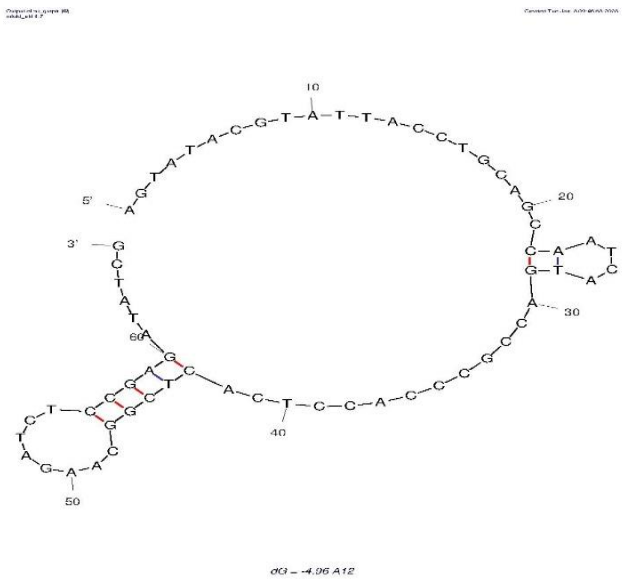

Figure 8: Visual representation of the optimal secondary structure for the A12 aptamer, as predicted by mFold algorithms. Sequence length = 66. 17 A's, 19 C's, 12 G's, 18 U/T's, and 0 N's. ΔG = - 4.96

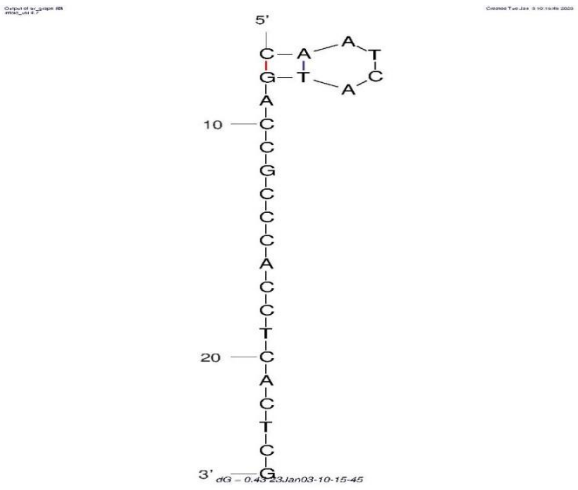

Figure 9: Visual representation of the optimal secondary structure for the TA12 aptamer, as predicted by mFold algorithms. Sequence length = 25. 6 A's, 12 C's, 3 G's, 4 U/T's, and 0 N's. ΔG = 0.43.

Table 5 summarizes the nucleotide sequences and the corresponding dissociation constants (K_D) for the aptamers used and their variants. The primer regions have been underlined. Notably, aptamer A12 exhibited a low K_D of 11.22 ± 1.3 nM, indicating the strongest binding affinity compared to others

Table 5: Nucleotide sequences and the corresponding dissociation constants (K_D) for the aptamers.

Aptamer	Sequence	KD (nM)
A10	AGTATACGTATTACCTGCAGC CCAACCCTATGCTTCAACGGTCTTTGCAAGATCTCCGAGATATCG	15.47 ± 0.39
TA10	CCAACCCTATGCTTCAACGGTCTTT	13.58 ± 2.3
A12	AGTATACGTATTACCTGCAGC CAATCATGACCGCCCACCTCACTCGGCAAGATCTCCGAGATATCG	11.22 ± 1.3
TA12	CAATCATGACCGCCCACCTCACTCG	16.58 ± 1.2

4. Discussion

Figure 2 shows the aptamer pool and how aptamers were selected to bind to the pathogen and sequenced. Test for binding against viable *A. acetic* cells was done by synthesis with fluorescent labels. After the aptamer pools were labelled with fluorescence, binding activity of 14 cloned aptamers was evaluated and fluorescence was measured as binding occurred, fluorescence intensity values are shown in Table 1.

Detection of aptamers and their binding potentials to microorganisms has been previously demonstrated by Davydova *et al.* (2016). Results from this study revealed some aptamers that are capable of actively detecting live *A. aceti* bacteria in beer. Table 1, 2 and 3 shows the binding activity of selected aptamers. The figures revealed that there are significant differences between candidate aptamers and their binding ability, as corroborated in reports obtained by Cho *et al*. (2010).

Fourteen aptamers were analyzed after SELEX. A10 and A12 were seen to bind strongly to the cells with the highest mean fluorescent intensity values of 150 and 180 with corresponding % of fluorescent cells (86 and 85), respectively. Some aptamers bound faintly to the cells, which indicates that not all aptamers will bind to the microbial strains if used (Siddiqui *et al.*, 2021). The least binding aptamers were A1, A2, A5, A6, A14 and A15, with fluorescent intensity values between 48 – 50. Values obtained for A10 and A12 binding activity revealed P values < 0.05, which shows that there is a significant difference between the means of these aptamers, which is in concordance with the results of Malatiji *et al.* (2022), who also reported a significant difference in fluorescence emission of aptamers used in the study.

The difference between the mean relative intensity values between different samples is noticeable. It is much higher in A12 (Table 4). A10 and A12 was subjected for further experiments since they exhibited the highest fluorescence intensity. It is important to investigate the specificity of aptamers even though they bind, to see if the primer sequences are responsible for selective binding (Lakhin *et al.*, 2013). Therefore, truncated sequences of the aptamers TA10 and TA12 (sequences without primer regions) alongside A10 and A12 were compared with *S. cerevisiae* and *Lactobacillus brevis* (non-target cells) to evaluate the significance of primers in their binding capabilities. Flow cytometry was used for this assessment (Cho *et al.*, 2010). 120 ± 3.8, 119 ± 5.2, 111 ± 6.2 and 110 ± 4.5 was the highest value observed by *A. aceti*. *S. cerevisiae* and *Lactobacillus brevis* had low values. The highest mean fluorescent intensity was observed only in *A. aceti*, which means that these aptamers are more specific to binding to *A. aceti* than the other organisms used. Siddiqui *et al.* (2022) also obtained a similar result and discovered aptamer E18R to be the best candidate among generated pool of aptamers in terms of binding and specificity to detect E. *coli* 157. Therefore, validating the selected aptamer from our result to be very specific.

It was observed that A10 and TA10 (110 ± 4.5, 111 ± 6.2) ; A12 and TA12 (120 ± 3.8 , 119 ± 5.2) are close in terms of their mean fluorescent intensity. A12 has the highest mean fluorescent intensity (120 ± 3.8). A12 exhibits a higher mean fluorescent value than A10, TA12 and TA10. Overall, this result suggests A12 as the best aptamer to detect *A. aceti* organisms in beer. This result also suggests that the aptamers would not bind to other bacteria because of their unique design just like Song *et al*. (2017) reported, and truncating the primer region might not affect its binding to the molecule of interest, justified by González *et al*. (2016), who reported that truncating an aptamer sequence is another method of eliminating nucleotide portions of the aptamer that are not involved in direct interaction with the target cells. Also, there was no significant difference observed for their binding specificity among the candidate aptamer which means that their inherent sequences are sufficient for their specificity.

The aptamers' binding affinity was visualized in table 5 and 6. From the sigmaplot graph, it can be observed that the fluorescence intensity increases with more concentration, this is consistent with results obtained by Li *et al*. (2021) and a recent study by Zhang *et al*. (2022). Similarly, the result agrees with the research done by Wang *et al*. (2014), which reported that the fluorescence intensity of an aptamer is directly proportional to the aptamer affinity; this means that it is normal for more cells to bind to the aptamer upon increasing the aptamer concentration. This implies that the fluorescence intensity can also be used to measure the target concentration, as described by Li *et al*. (2021). In contrast, after 100 nM concentration for A10 and A12, the % fluorescence cells do not seem to increase at the same rate as the initial concentration of 10 and 25 nM (a different doubling rate), which indicates that there is no dose-dependent variation between aptamers concentration and fluorescent intensity. Similar findings were also reported by Li *et al*. (2021), showing how fluorescence intensity peaked at 500 nM in a concentration-dependent relationship before decreasing. However, it is worth mentioning that the study by Li *et al*. (2021) used RNA oligonucleotides, amongst other different methods. The fact that aptamer concentration does not matter for detection as long as it binds to its targeted cell is consistent with previous studies.

According to Takenaka *et al*. (2017), there is a relationship between the dissociation constant and the affinity of the target molecule which can also be calculated with thermodynamic stability. The KD values in table 7 were used to quantify the affinity and binding ability an aptamer has between molecules of interest. A strong bind of an aptamer with another molecule needs a low KD. Therefore, the lower the KD values, the stronger the affinity and binding activity, and vice versa. A12 had the lowest KD value of 11.22 ± 1.3 (nM), and A10 with 15.47 ± 0.39. KD values in truncated aptamers were higher for TA12 and TA10 (16.58 ± 1.2 and 13.58 ± 2.3), respectively. From all indications and analysis, A12 is the best aptamer from the pool to detect *A. aceti*.

Table 7 shows the nucleotide sequences of successfully sequenced aptamers. They were further analyzed for their secondary structures using the mFold software. The secondary structure of aptamer molecules has been reported to help in protein-DNA recognition (Sullivan *et al.*, 2019). The best folding structure/sequence (Figure 4 to 7) of the candidate aptamers that can be formed in a buffer was determined by the mFold software by evaluating the Gibbs energy change. The truncated aptamers revealed higher ΔG values than their original sequence which demonstrates the importance of the primer sequence. As explained by Sorokina *et al.* (2022), the aptamer structure with the least Gibbs free energy was predicted to be the best according to the thermodynamics principle for protein folding; (A10: ΔG = -4.40, TA10: ΔG =

0.07, A12: ΔG = - 4.96, TA12: ΔG = 0.43). Conserved moieties were also observed for both truncated and original aptamers which might explain aptamers specificity and binding i.e why truncated sequences could still bind to target cells. Furthermore, energy dot plots and structural dot plots for thermodynamic change and structural comparison among aptamer structures were generated from the mFold software.

4. Conclusion

Aptamer based biosensor technology has gained attention recently because of its timely, economic and efficient approach in producing reliable results than other methods. A12 was revealed to be the best aptamer from the pool, with significant differences when compared with other candidates. Adding ten µM of A12 aptamer was enough to bind and produce detection. Adding more aptamers will increase the fluorescence produced.

This aptamer can therefore be applied to detect target organisms by electrical or optical sensation. They can also be further optimized in terms of concentration and efficiency. A future projection in this study would be to explore the combination of A10, A12 and nanoparticles to see how detection can be further increased against spoilage organisms. Engineering these aptamers might also allow for the detection of other microorganisms in beer.

Declarations

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Conflicts of interest

The author declares no conflict of interest

Ethics approval

Not applicable

Ethics/Consent to participate

Not applicable

Consent for publication

Not applicable

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