Selection of DNA aptamers to Salmonella Enteritidis and Salmonella Typhimurium

By

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Abstract

Aptamers are single stranded oligonucleotides that forms 3 dimensional structure and binds to target with high affinity and specificity. The target that aptamers can bind to is vast. In this project, the aptamers selected for two serotypes of Salmonella bacteria that are causes a widespread of food borne illness (Salmonella Enteritidis (SE) and Salmonella Typhimurium (ST)). These aptamers were selected using the cell SELEX method and have high binding affinity to the target bacteria. The pool of aptamers were select in binding. The SE aptamer will only bind the SE bacteria and the ST aptamers will only bind the ST bacteria. The highest binding affinity pool (selected using flow cytometry) was cloned and sequenced. Further studies were done on the effect of the these aptamers on SE and ST bacteria. We were able to find antibacterial effect with the clones and aptamer pool against SE and ST bacteria.

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Statement of contribution

The aptamers for Salmonella bacteria cells (ST and SE) were selected by Anna G. Savitskaya in Krasnoyarsk, Russia. The cloning was done at the University of Ottawa, Dr. Maxim Berezovski's lab under the supervision of Dr. Anna S. Zamay and the help of Darija Muharemagic.

Introduction

Among world's vast amount of pathogens, Salmonella bacteria is one of the most dangerous pathogens for humans. Despite the increase in drug research, salmonella caused illness is still one of the most difficult infections to treat. Salmonella is a rod-shaped gram-negative motile enterobacteria. There are many different strains of Salmonella bacteria that cause diseases. The organism is associated with 26% of all food borne illness leading to hospitalization. Salmonellosis is the infection of humans by Salmonella bacteria that contains multidrug resistant strains. Salmonella lives in the intestinal tracts of humans and other animals. It gets transmitted to humans usually by the consumption of feces contaminated food [2]. The symptoms of Salmonella infection include diarrhea, fever, and vomiting. The common treatment for salmonella infection is the intake of antibiotics such as ampicillin, trimethoprim-sulfamethoxazole. However, some Salmonella bacteria subspecies developed drug resistance to the antibiotics. This was caused by the use of antibiotics to promote the growth of the animals. The most common widespread Salmonella serovars belongs to the *Salmonella Enterica* subspecies. Out of the *Salmonella Enterica* subspecies, *Salmonella Enteritids* (SE) and *Salmonella Typhimurium* (ST) cause the most concern due to its resistance to antibiotics [1].

Salmonellosis infection often happens due to ingestion of Salmonella contaminated food. However it can also be transmitted from domestic animal (pets) to humans. The evolution of Salmonella bacteria serotypes is mainly caused by intensive animal husbandry. In 1992, an outbreak of Salmonella Enteritidis infection broke out in Europe. During that time, 95% of the cases dealt with Salonella Enteritidis (SE) and only 5% of the cases were the infection of Salmonella Typhimurium [6]. However, studies

have shown that recently, there was an increase in the circulation of serotype Salmonella Typhimurium. Therefore, it is important that theses two serotypes are studied and distinguished. The other most important aspects about theses Salmonella bacteria are its high resistance to many modern common antimicrobials, and disinfections. Antimicrobials especially a group of fluoroquinolones are widely used for the optimal treatment of Salmonellosis. Resistance to these drugs was caused by the mutation in bacterial genome. Study shows that some Salmonella serotypes also developed drug-resistant gene in their genome. The multi drug resistance bacteria are now harder to control creating a severe limitation to the treatment of infections.

Salmonella Enteritidis is known for its ability to penetrate unhatched eggs, passed from mother hen. Many infections were related to the consumption of raw eggs, and uncooked poultry products derived from contaminated intact shell eggs. Studies have identified a gene in SE that benefit its survival in albumen and plays an important role in the contamination of intact shell eggs. Salmonella Typhimurium is a virulent strain that is resistant to many antibiotics. The spread of ST was linked to the intensive use of antibiotics in human medicine and agriculture. ST bacteria are mainly associated with cattle and pigs. Since the two types of Salmonella bacteria are the most common types that cause infections, they were studied and new methods are developed for the treatment and detection of the Salmonella infections [7].

Recently, a novel class of drugs based on low immunogenic high specific synthetic ssDNA or ssRNA aptamers are currently in clinical development as treatments for a broad range of common diseases. Aptamer are very short single stranded oligonucleotide, usually 80-100nt long, composed of DNA or RNA. The single stranded

oligonucleotide will form 3 dimensional structures, depending on their sequence, and recognize and bind to the target molecule. It is important to note that aptamer bind to a variety of target molecule ranging from small organic compound to antibiotics, peptides and even whole cells. Aptamers are comparable to antibodies in their dissociation constant and their ability to bind to target molecule with high specificity and high affinity. However, aptamers exhibits many interesting features and advantages that set them apart from antibodies. The stability of Aptamers, especially DNA aptamers is almost infinite. Furthermore, they can be renatured after denaturation. It is also very cheap and easy to produce and there is very little batch-to-batch variation. The most interesting feature about aptamers is that they are selected entirely *in vitro*, without the use of animals. Importantly, selected aptamer have little to immunogenic properties. Due to the high binding affinity of aptamers to its target, it has become a useful to tool for diagnosis and therapeutic applications [2].

Aptamers are generally selected from a large pool of random single stranded oligonucleotide library by a process called SELEX (Systematic Evolution of Ligands by Exponential Evolution). Some of the oligonucleotide in the vast library is anticipated to bind to the target molecule with high affinity. There are two general steps in a SELEX process: the partitioning of binding and nonbinding aptamers, and the amplification of binding aptamers to made ready for another round of selection. Usually, 6-12 rounds of selections are made to select one specific target. A variety of modified SELEX protocols such as cell-SELEX were developed to increase the specificity of aptamer selection. In the case of cell-SELEX, a pool of aptamers was first exposed to a line of cell that does not contain the desired target [1]. The aptamer that binds to these targets are considered

non-specific binding aptamers and were eliminated. The remaining aptamers were treated with target cells, and the aptamers that was bound to the target was collected and amplified and treated for another round of selection. This type of selection is called negative selection. The idea of SELEX is the Darwinian principle, survival of the fittest. The apatmer populations that remained during later selection have the highest binding affinity to the target. These specific aptamers can be used for receptor activation, biomarker discovery and recognition, and intracellular protein localization [3].

In this project, aptamers were selected for the surface biomarkers specifically to live Salmonella Enteritidis and Salmonella Typhimurium bacteria cells. The library used for aptamer selection is 80nucleotides long with 40 randomized nucleotides and known 5' and 3' primers that are 20nucleotides each. The primers are fluorescently labeled and used for the amplification of selected aptamers. The fluorescent dye used for labeling is Alexa -488, which has an emission wavelength of 514nm. The main goal of the project is to distinguish between two types of Salmonella serotypes (SE and ST). The selection of aptamers was based on the different type of surface markers present on SE and ST. It is interesting to realize that there are many complex and convoluted targets on the cell surface, many of which cannot be determine. Subtle differences between two types of cells can be enough to differentiate and categorize. The aptamers selected for SE and ST can be used as a diagnostic tool for SE and ST infections. These aptamers can also be used as a vehicle for drug delivery. The use of live bacteria cells is also important comparing to the selection with specific protein markers.

There are two parts to the selection of aptamers that bind to SE or ST. The first part is the finding / development of a method that will give the highest binding affinity

for aptamer to their target. Also a method that is able to distinguish the two different Salmonella bacteria (the binding of one and not the other). The second part is to test the specificity of these aptamers, the challenges associated with selected aptamers as a diagnostic tool and other effects of these aptamer pools. One of the challenges with aptamers is that it has a very short half-life in the body. Since aptamers selected are composed of ssDNA nucleotides, there is a high chance of degradation by nucleases in the body. However, aptamer can be selectively modified thus protected from the effects of nucleases. Recent studies have shown that modification at the 2' ribose sugar will increase the half-life of aptamers in the body.

Preliminary studies have shown that these SE, ST specific aptamer also exhibits antibacterial effects. Studies have shown that SE/ST cells that were incubated with its respective aptamers have grown less colonies comparing to SE/ST cells without aptamers. Further studies are required to determine this phenomenon. This investigation of antibiotic effect of aptamer to Salmonella bacteria is contributing the development of therapeutics that control multi-drug resistant bacteria strains [2].

Material and Methods

DNA aptamer library:

N40 randomized DNA library was used for all experiments. The selection started with a naïve ssDNA library (integrated DNA technology, USA). The library consists of a random region of 40 nucleotides flanked by two constant primers, which are 20 nucleotides each. 1uM of DNA library was used for aptamer selection ($6x10^{13}$ sequences). Since the pool is randomized, it allows a large diversity of potential aptamer

that can bind to the target. The constant primers were used for the amplification of potential aptamers. The primer-hybridization site is consist of the following 5' - CTC CTC TGA CTG TAA CCA CG N40 GC ATA GGT AGT CCA GAA GCC - 3'.

Selection of Aptamer using modified SELEX:

Salmonella Enteritidis (SE) and *Salmonella Typhimurium* (ST) were isolated in Krasnoyarsk region of Russia. Aptamers were selected separately for the two types of salmonella bacteria. The first aptamer selection round was selected as a posiive selection. Only the target salmonella bacteria cells were used. Prior to selection, $3x10^7$ bacteria were washed in DPBS (Dulbecco's phosphate buffered saline, Sagma-Aldrich, USA). The randomized DNA library above was incubated with washed bacteria for 30min at 25^oC and then centrifuged at 3,500x g for 10 min at 25^oC. To remove the unbound aptamers, the pellet was washed twice with DPBS. For the release aptamer from the bacterial surface, the pellet was re-suspended in 95uL of 10mM Tris-HCl buffer containing 10mM EDTA, pH7.4 (TE) buffer and heated for 10min at 95^oC. After denaturation of bacteria and the release of bound aptamers, the solution was centrifuged at 14,00 x g for 15min at 4^oC. The supernatant, which contains aptamers were collected and the pellet or bacterial debris was discarded. The aptamers were stored at -20^oC freezer in order to avoid denaturation.

Negative selection:

Other non-target bacterial cells were selected for the procedure of negative selection. For the negative selection against Salmonella Enteritidis, the bacteria mixture included, Salmonella Typhimurium (Krasnoyarsk), Staphylococcus aureus (The American Type

Culture Collection (ATCC 25923)), Escherichia coli (ATCC 25922), pseudomonas aeruginosa (ATCC 27853), and clinical stain of Citrobacter freudi was used.

For the negative selection against Salmonella Typhimurium, the bacteria mixture included, Salmonella Enteritidis (Krasnoyarsk), Staphylococcus aureus (The American Type Culture Collection (ATCC 25923)), Escherichia coli (ATCC 25922), pseudomonas aeruginosa (ATCC 27853), and clinical stain of Citrobacter freudi was used. In the case of negative selection, 100nM ssDNA aptamers were first incubated with the negative bacterial mixture for 30min at 25^{o}C and then centrifuged at 3,500 x g for 10min at 25^{o}C. The supernatant this time was kept and used for positive selection described above.

Preparation of enriched DNA library:

Each pool of aptamers were collected and amplified after each round of selection. The aptamer library used for the SELEX round selection was obtained from previous round's aptamer pool. Before each round of selecting and binding experiments, the ssDNA libraries and aptamer pools were denatured. The aptamers were heated for 5min at 95^{o}C in DPBS (Sigma-Aldrich, USA) and then re-natured on ice for 10min.

Polymerase Chain Reaction (PCR):

Polymerase chain reaction was used for the amplification of aptamer pool after selection. A(n) symmetric and asymmetric PCR was done for the analysis of each pool. For symmetric PCR, 5ul of selected aptamer pool in TE was mixed with 45ul of symmetric mastermix. The symmetric matermix contains 1x PCR buffer, 25uM $MgCl_2$, 5U uL^{-1} Taq Polymerase (Syntol, Russia), 10mM dNTPs, 0.5uM forward primer (5'- CTC CTC TGACTG TAA CCA CG -3'), 0.5uM reverse primer (5' GGC TTC TGG ACT ACC

TAT GC -3')(Syntol, Russia). For asymmetric PCR, the same procedure was used except for the primers. 1uM of forward Alexa-488 primer (5' – Alexa488- CTC CTC TGA CTG TAA CCA CG-3') and 50nM of revere primer (5' GGC TTC TGG ACT ACC TAT GC -3') were used. The polymerase procedure was performed first with preheating of aptamer at 95^{o}C for 2min followed by 15cycles of denaturing at 95^{o}C for 30s, primer annealing at 56.3^{o}C for 15s, and elongation at 72^{o}C for 15s. The final temperature of PCR was set and held at 4^{o}C. The concentration of the PCR product was analyzed using 3% agar gel electrophoresis. The amplified aptamers were fluorescently labeled with Alexa-488 and analyzed in gel-documenting system GBOX/EF2-E. The evolved aptamers were stored at -20^{o}C

Flow Cytometry and Fluorescence Microscopy:

FC-500 flow cytometer (Beckman Coulter Inc., USA) was used to determine the affinity of the evolved aptamer for SE and ST against ST and SE. 0.5x10^{6} SE or ST bacteria were preincubated with 1mg mL^{-1} of Salmon sperm DNA for 15min at room temperature. The pretreated SE or ST were incubated with 100nM Alexa-488 labelled aptamers respectively in 500uL DPBS buffer for 30min at 25^{o}C. Each sample was washed once to remove unbound DNA and resuspended in 0.5mL of DPBS. The solution was measured by flow cytometer, and 100,000 events were recorded. The gate for intact baterial SE and ST in DPBS was chosen and used as a negative fluorescence. Data analysis was performed using CXP cytometer

Fluorescence microscopy was used to measure the affinity of the evolved pools against SE and ST. Probes were prepared the same way as Flow cytometry. The fluorescence was analyzed under microscope.

Cloning and Sequencing:

The highest binding affinity pools were cloned and sequenced. Promega cloning kit was used for the cloning of aptamer pools. E. coli bacteria cells were used to transform aptamers into the bacteria DNA. The petri dish contained the following media, purified agar, LB broth, X-gal and Ampicillin. The double stranded DNA aptamer was made from ssDNA aptamers and purified using gel purification. After the plating of E. coli bacteria cells, it was incubated at 37^{o}C for 24hrs. The colonies that contained inserts were collected and added to 3ml of LB media. The cells, were incubated again at 37^{o}C for 24hrs. 5uL of bacteria cells were added to PCR mastermix and sent out sequencing

Evaluation of antibacterial effect of aptamers on ST and SE:

One day bacterial culture in nutrient broth of SE and ST (10^3 cells/mL) were incubated with high binding affinity aptamer pools, clones, and ssDNA library in final concentration of 1uM. Intact ST and SE cells were used as a control. All samples were incubated for 30min at 25^{o}C. The mixture was incubated for 24hrs at 37^{o}C. The colonies were counted and recorded.

Results and Discussion

Optimization of selection protocol:

The first important task is to determine the best / optimal selection method for the selection of aptamers against the two live Salmonella bacteria cells (Salmonella Enteritidis (SE) and Salmonella Typhimurium (ST)). As outlined in the material and method section, there are two different steps to SELEX (systematic evolution of ligands by exponential enrichment): amplification and purification. However, the methods needed to be modified in order to meet the requirement for this project. The main focus of the selection process is the ability to select aptamers that are specific to SE and specific to ST. The DNA library used for the selection contains $6x10^{13}$ different sequences. There is a high possibility of non-specific binding aptamers present in the selection pool. Also there is also a possibility that the aptamers that binds to SE will also bind to ST. Therefore, in order to increase the specificity of these aptamers, a modified SELEX procedure was used to answer these challenges. The selection of aptamers toward live SE and ST were selected by cell – SELEX method. This method is an *in Vitro* selection of oligonucleotide binders to live bacteria cells from a random single stranded (ss) DNA library. This library of aptamers contains 40 random nucleotides (nt) flanked with 20nt 5' primer and 20nt 3' primer. A negative selection was added to the SELEX process to increase specificity of the aptamers selected against SE and ST. Figure 1 outlines the general procedure that was adopted for rounds 2-12 of selection.

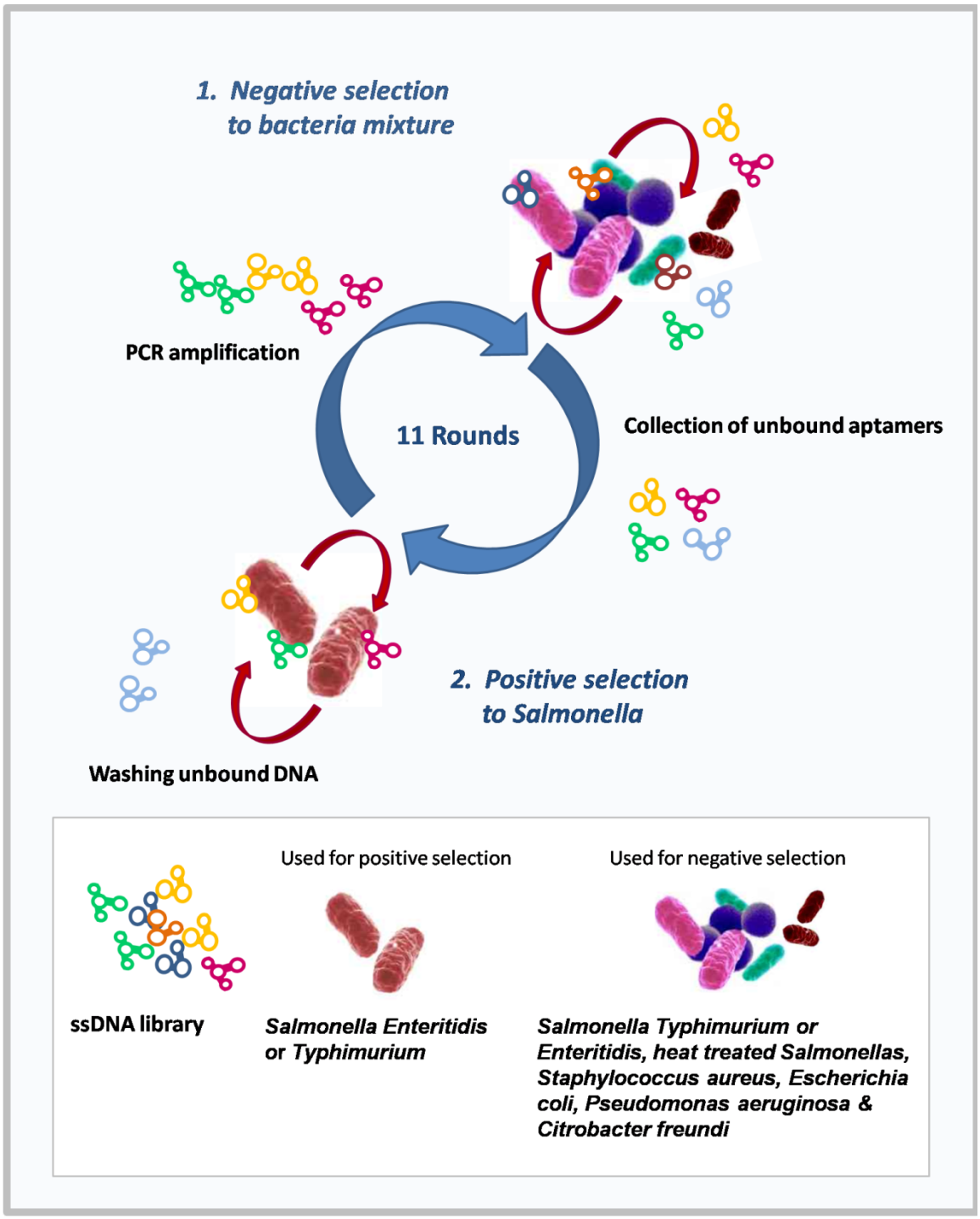


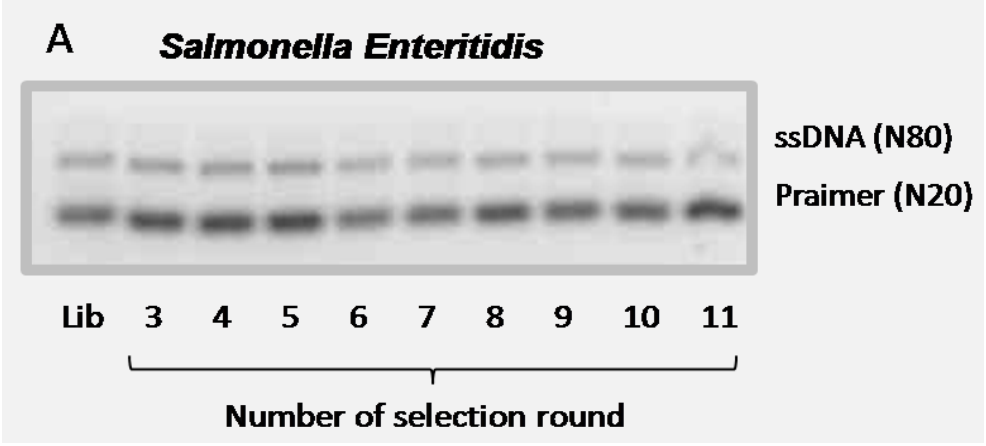
Figure 1. Schematic representation of aptamer selection towards Salmonella Enteritidis and Salmonella Typhimurium. The first selection round included just positive selection to Salmonella Typhimurium or Enteritidis. The second round and each subsequent one was started from the negative selection and consisted of following steps: incubation with bacteria mixture: Salmonella Typhimurium or Enteritidis, heat treated Salmonellas, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Citrobacter freundi; collection of unbound sequences; incubation of collected ssDNA with

Salmonella Enteritidis or Salmonella Typhimurium; washing of unbound ssDNA from salmonella; extraction of bound ssDNA sequences; ssDNA amplification by symmetric and asymmetric PCR.

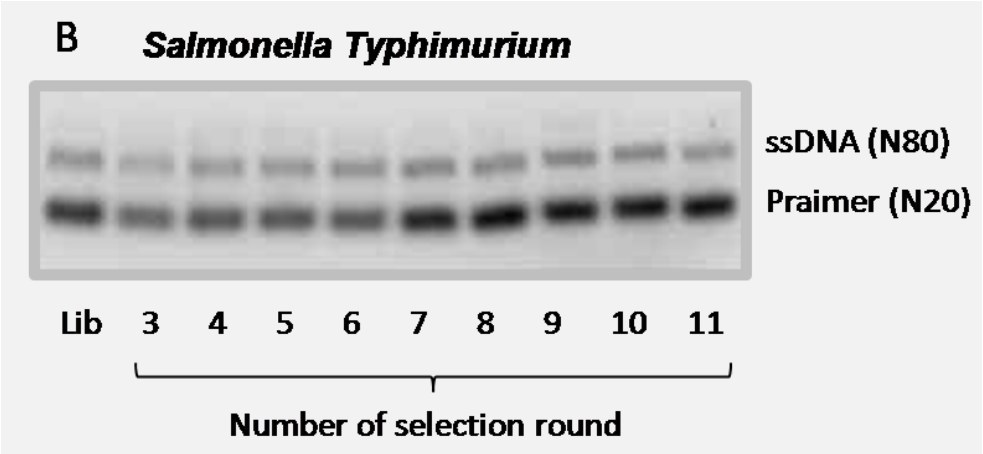
It is important to note that 2 pools of ssDNA library were used for selection. The first pool was used for the selection of SE and the second pool was used for the selection of ST. The two selections have the same procedures but were selected independently. Also, all rounds of selection were performed at room temperature. For the first round of selection, no negative selection was done. Starting with an initial pool of the N40 DNA library, a general SELEX procedure was used for the selection of SE/ST aptamers. The random ssDNA library was incubated with SE/ST, followed by the washing of unbound ssDNA from the bacteria cells. The bound DNA was then extracted and amplified by symmetric PCR followed by asymmetric PCR. The asymmetric PCR product was used for the next round of selection. As outlined above, round 2-12 started with a negative selection, a necessary step since the aptamers bind to a wide variety of molecules that are not specific to the target. The process starts with the initial incubation of negative selection bacteria cell mixture to aptamers obtained from the previous round. The mixture include SE (if selecting for ST aptamers), ST (if selecting for SE aptamers), heat treated Salmonellas, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Citrobacter freundi. The aptamers that were in the supernatant (those that did not bind to the bacteria mixture) was collected and incubated with SE/ST for positive selection. The aptamer that was bound to SE/ST was collected and amplified by symmetric PCR and asymmetric PCR before it was used for successive rounds of selection. In total, 12 rounds of selection was done and analyzed before cloning and sequencing.

Completion of initial rounds of aptamer selection:

Selection using the modified SELEX method (outlined above) was done for each round. Aptamers were incubated at 25^{o}C with the live SE and ST bacteria cells. For each round, the bound aptamers were analyzed and verified by gel electrophoresis. Figure 2 shows the electroforegram of ssDNA PCR products (asymmetric products) from different selection round of Salmonella enteritidis and Salmonella typhimurium. The gel electrophoresis was done to determine the concentration of aptamer present. The gel can also separate different contaminations in the aptamer pool. If a contamination is present in the pool, purification procedures were used to purify the aptamers base on the size. It is very important to determine the concentration of aptamers amplified. It is ideal to add the same concentration of aptamers to similar amount of live SE/ST cells. As shown in Figure 2, the concentration of aptamer in each pool is about 100nM. This concentration is sufficient for selecting aptamers. The top band represents the aptamer (N80) that is present in the pool, and the bottom band represents the primer (N20). When there is insufficient amplification present in the aptamer pools, additional asymmetric PCR was done. The amplified aptamers were mixed together and concentrated to a lower volume. The concentrated aptamers were used for the next round of selection. Twelve rounds of selection were completed, leading to the identification of highly specific pool of aptamers that bind specifically to SE/ST. However, each aptamer pools will have different binding affinities.



(a)



(b)

Figure 2. Electroforegram of ssDNA PCR-products (aptamer pools) from selection rounds 3 to 11. The ssDNA library was used as a positive control. The top band represent the ssDNA aptamers at 80 nucleotides. The bottom band represent the primer band at 20nucleotides. Similar amount of aptamers were incubated with SE and ST cells for consistent results. Salmonella Enteritidis (A) and Salmonella Typhimurium (B).

Verification of aptamer binding:

After the completion of aptamer selection, assays were done to determine if the aptamers were indeed binding to the salmonella bacteria. As mentioned above, the 5' primer of aptamers were fluorescently labeled with Alexa -488. The emission wavelength of Alexa – 488 is at 514nm. Therefore the binding affinity should be measured using a fluorescent signal. Flow cytometry method was chosen to determine the binding affinity of aptamer pools to the SE/ST bacteria. The fluorescently labeled pools of aptamers were incubated with Salmonella Enteritidis or Salmonella Typhimurium accordingly. The cells were analyzed by flow cytometryusing FC-500 flow cytometer (Beckman Coulter Inc., USA). The principle behind flow cytometry is that, when the fluorescently labeled aptamers bind to the target cell, the fluorescence intensity will increase. The intensity level is also proportional to the amount of aptamers that was bound to the cell. The forward scattering and the fluorescent intensity were measured for different pools.

The ST and SE bacteria were initially treated with 1mg/mL of Salmon sperm DNA. Salmon sperm DNA was used as a masking nucleic acid to suppress non-specfic binding. Intact SE and intact ST were first measured, 100,000 events were recorded. It was found that when intact SE and ST cells ran on its own, the cells were detectable in flow cytometry. Therefore, a threashold level was set outlining where the auto-fluorescence of the bacterial cell fell. The gate for intact bacteria SE and ST in DPBS was taken as a negative fluorescence. All other fluorescence measures were based on the negative fluorescence.

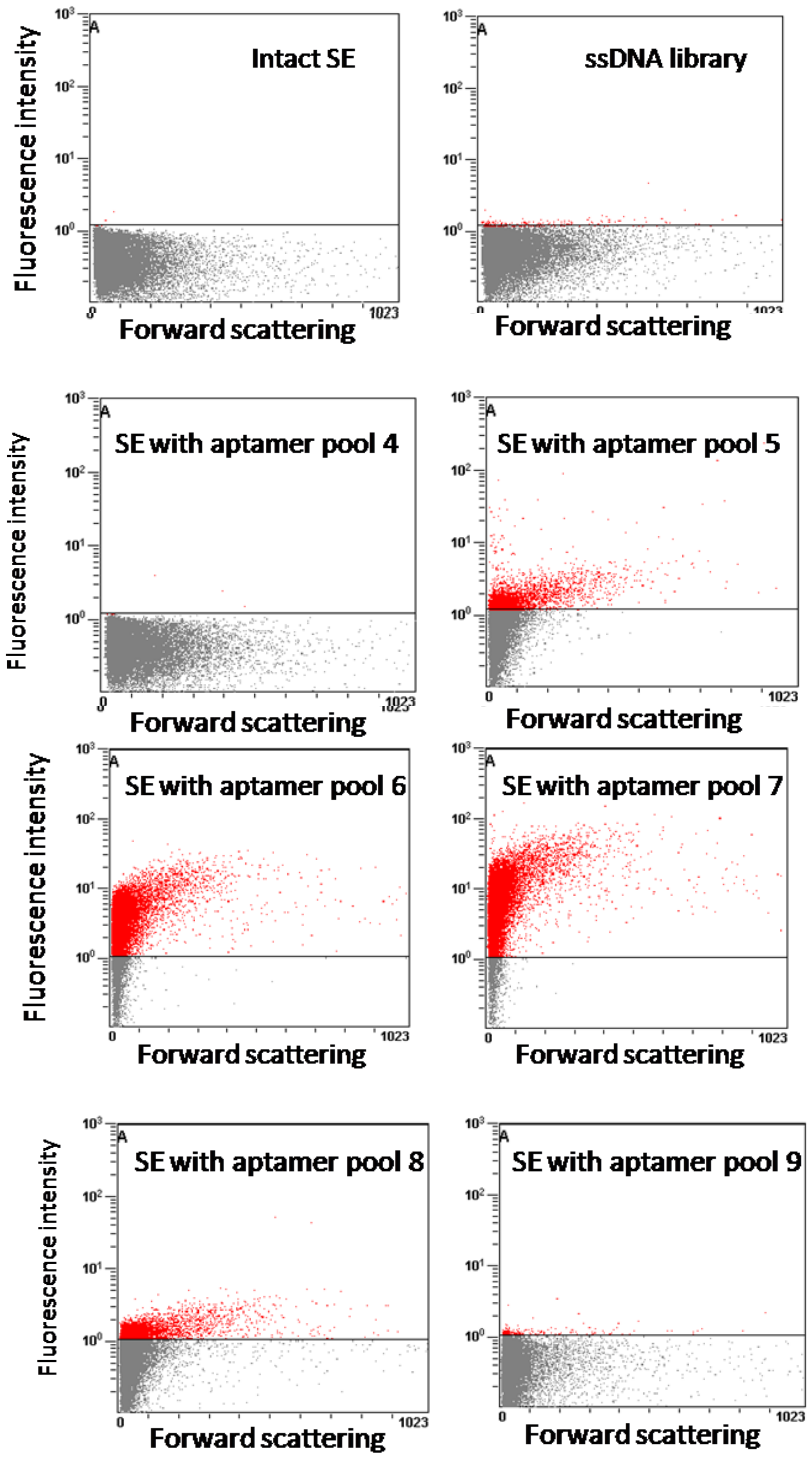


Figure 3a. Flow cytometric analysis of selected aptamer pools affinity to Salmonella Enteritidis. The intact SE fluorescence intensity as used as a negative fluorescence. All florescence intensity above the threshold line exhibit binding properties. Red indicates cells are bound to the aptamers.

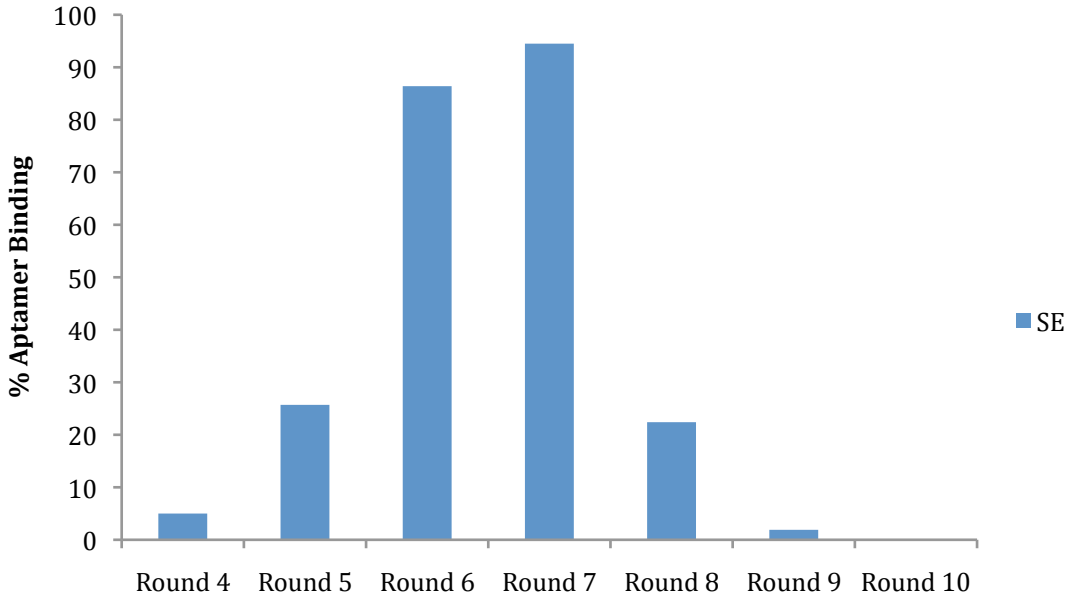


Figure 3b. The percentage binding of aptamers to Salmonella Enteritidis bacteria cells. Round 7 contains the highest binding percentage compared to the rest of the rounds. Round 7 was cloned and sequenced for further analysis

From figure 3a, it can be seen that pools 5, 6, 7, 8 SE aptamers all have higher fluorescence intensity than negative fluorescence. The fluorescence above negative gate was considered to be the real fluorescence. This means that Alexa-488 labeled aptamers were bound to the bacteria. Fluorescence intensity was measured for ssDNA library as a control. The ssDNA library showed little to no fluorescence. This makes sense because, the ssDNA library is random and the bindings to the target bacteria cells are not specific. As shown in figure 3a, Pool 7 has the highest fluorescence intensity. This means that the pool has the highest amount of aptamers that bind to the target SE cell. The binding percentages of aptamers to SE cells were calculated. An increase in the binding affinity was observed in figure 3b. The graph shows the percentage binding of SE aptamers to SE bacteria cells found from round 4 to round 9. The population expressing Alexa-488 fluorescence and binding affinity was observed over rounds 4 (5% binding), 5 (25.7% binding), 6 (86.4% binding), 7 (94.5% binding), 8 (22.4% binding), and 9 (1.9% binding). From these percentages, it can be concluded that for SE bacteria cells, pool 7 SE aptamers have the highest binding affinity.

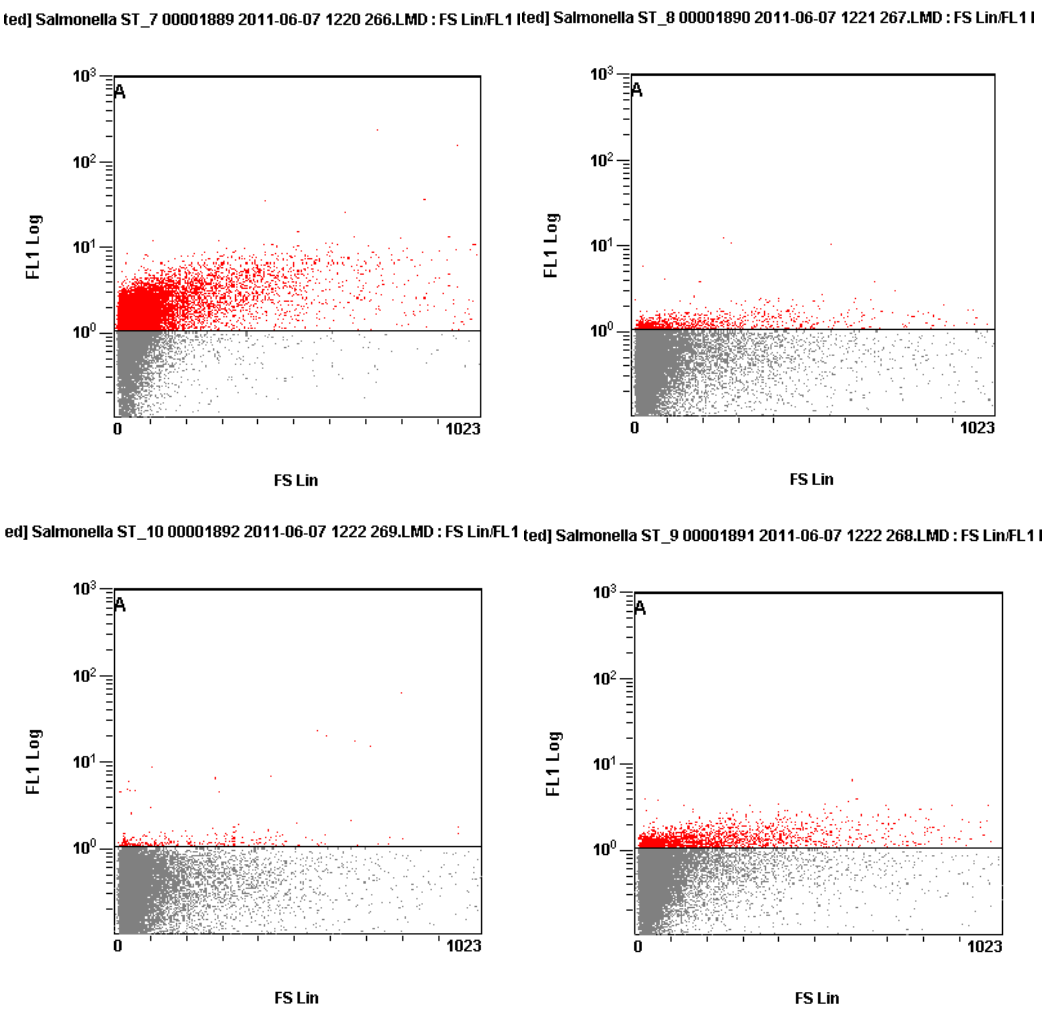


Figure 4a. Flow cytometric analysis of selected aptamer pools affinity to Salmonella Typhimurium. The intact ST fluorescence intensity as used as a negative fluorescence. All florescence intensity above the threshold line exhibit binding properties. Red indicates cells are bound to the aptamers.

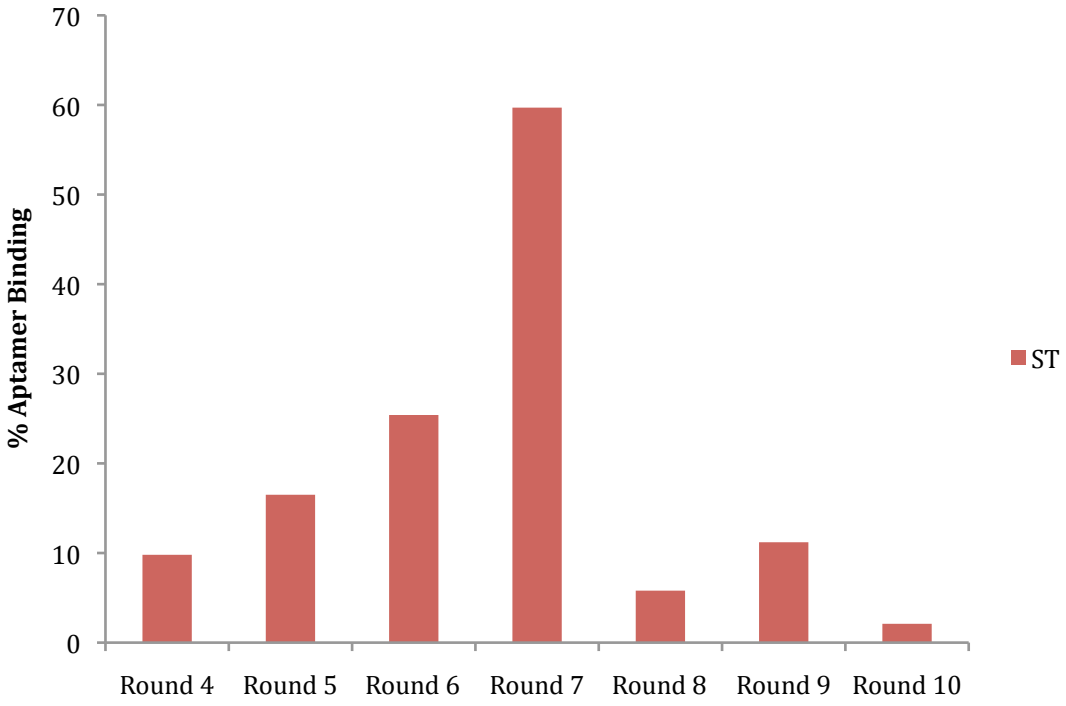


Figure 4b. The percentage binding of aptamers to Salmonella Typhimurium bacteria cells. Round 7 of ST aptamer pool showed the highest binding percentage. The aptamers from pool 7 was cloned and sequenced and used for future studies.

Same procedure analysis was done for ST binding aptamers. The SE and ST aptamers were selected simultaneously using the same protocol. However the aptamers selected are specific to each salmonella bacteria. The flow cytometric analysis of aptamer pool 7-pool10 affinity to Salmonella Typhimurium (ST) were observed. The fluorescence intensity was plotted against forward scattering. The fluorescence intensity as explained above, showed the binding affinity of aptamers to ST bacteria. The forward scattering indicates the size of the cell. Pool 7 ST aptamers have the highest amount of cells that exhibit high fluorescence intensity. The percentage binding was also calculated for the different aptamer pools (figure 4b). ST aptamers also expresses Alexa-488 fluorescence and binding affinity was observed over rounds 4(9.8% binding), 5 (16.5% binding), 6 (25.4% binding), 7 (59.7% binding), 8 (5.8% binding), 9 (11.2% binding), and 10 (2.1% binding). The highest aptamer binding pool is pool 7 ST aptamers. Therefore pool 7 SE aptamers and pool 7 ST aptamers were cloned and sequenced for further investigation.

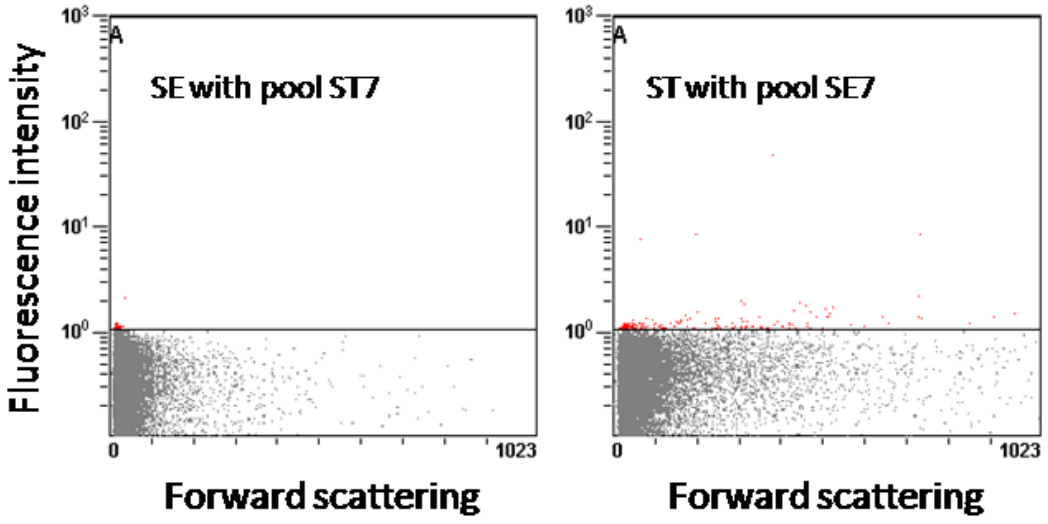


Figure 5. Flow cytometric analysis of selected SE aptamer pool 7 affinity to Salmonella Typhimurium and selected ST aptamer pool 7 affinity to Salmonella Enteritidis. The intact ST and SE fluorescence intensity as used as a negative fluorescence. All fluorescence intensity above the threshold line exhibit binding properties. Red indicates cells are bound to the aptamers.

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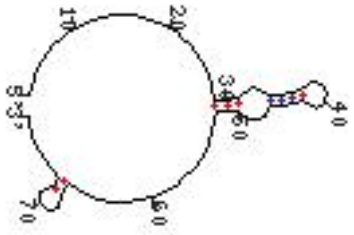
One of the most important aspect of this project was the selection of aptamers that were specific to one type of bacteria cell and not the other. The SE bacteria cells were incubated with the pool 7 ST aptamers for 30min, and the SE bacteria cells were incubated with the pool 7 SE aptamers for 30min. The unbound DNA was washed and the sample was resuspended in DPBS. 100,00 events were recorded. The gate for intact SE and ST bacteria cells were taken again as negative fluorescence. The fluorescence results showed that there are little fluorescence intensity increase (Figure 5). This means that pool 7 ST aptamers do not bind well to the SE bacteria cells. Also, pool 7 SE aptamers do not bind well to the ST bacteria cells.

Cloning and sequencing:

Pool 7 SE aptamers and pool 7 ST aptamers were cloned and sequenced. The aptamers were first purified using gel purification. This procedure decreases the presence of primers in the aptamers pool. E. coli cells were used for cloning. The aptamers were sent to McGill for sequencing. The results were analyzed.

SE1

Ctcctctgactgtaaccacgataatcaaggcaaaactaggggtttcgtagccatcctttcgcataggtagtccagaagc



SE 22

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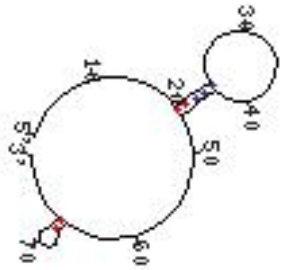
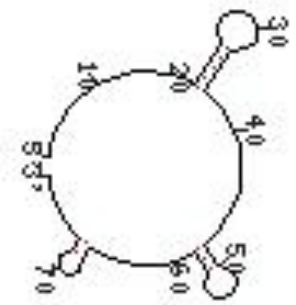


SE 34

ctcctctgactgtaaccacgatcattagattctgatctacgggtctactgcttattcaggcataggtagtccagaagc

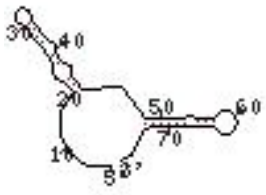


Three of the 20 sequences for SE aptamer pool 7 was selected and analyzed. The highlighted region of the sequence are the forward and reverse M13 primers. The nucleotides in the middle are specific for the binding of SE bacteria cells. The diagrams underneath the cloning sequences are the likely conformation of aptamer shapes. There are some similarities that is displayed between theses clones. The clones all exhibit one big round geometry that allows binding to the surface of the SE bacteria. The specific sites that these aptamer binds to have not been determined. However further studies will be done to determine the specific protein binding sites.

ST aptamer pool 7 was also sequenced and analyzed. The aptamers were also cloned using E. coli cells. Three sequences were selected to determine the similarity in structure.

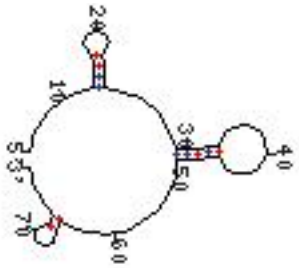
ST 6

Ctcctctgactgtaaccacggcctctaaggctcaccttgaagcgcccggactaacctgctgcataggtagtccagaagc



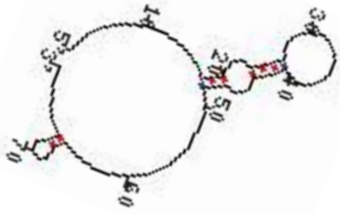
ST12

Ctcctctgactgtaaccacggtggtttgatcactattgggcctttgtgatgtcggtagtgcataggtagtccagaagcc



ST22

Ctcctctgactgtaaccacggcgccttactgactgcacgaggctaacgtcctcccttaatgcataggtagtccagaagcc



The highlighted region of the sequence are the forward and reverse M13 primers. The nucleotides in the middle are specific for the binding ST bacteria cells. The diagrams underneath the cloning sequence are the likely onformation of aptamer shapes. All three ST clones have a slander conformation compared to SE clones. The linear part of the ST clones could be the binding region to the ST cells. There is also a significant difference in the aptamer shapes between SE and ST aptamers. Therefore, the flow results shown in figure 5 can be justified.

Antibacterial effect:

Further studies were done to determine the effect of these aptamers. It was found that the aptamers and clones were able to decrease the amount of colonies that were grown on the petri dish. Three aptamer sequences from each pool of SE and ST were chosen for the further investigation of antibacterial effect. The antibacterial effect of aptamer pools and clones were estimated by the differences in the number of DE or ST colonies gown in petri dishes. There were two group of samples analyzed. The first group is the control samples (SE and ST bacteria in nutrient broth). The second group is the experimental samples (SE and ST bacteria in nutrient broth supplemented with 1uM of SE and ST aptamer pool respectively). Other experimental samples were analyzed with clones instead of aptamer pools.

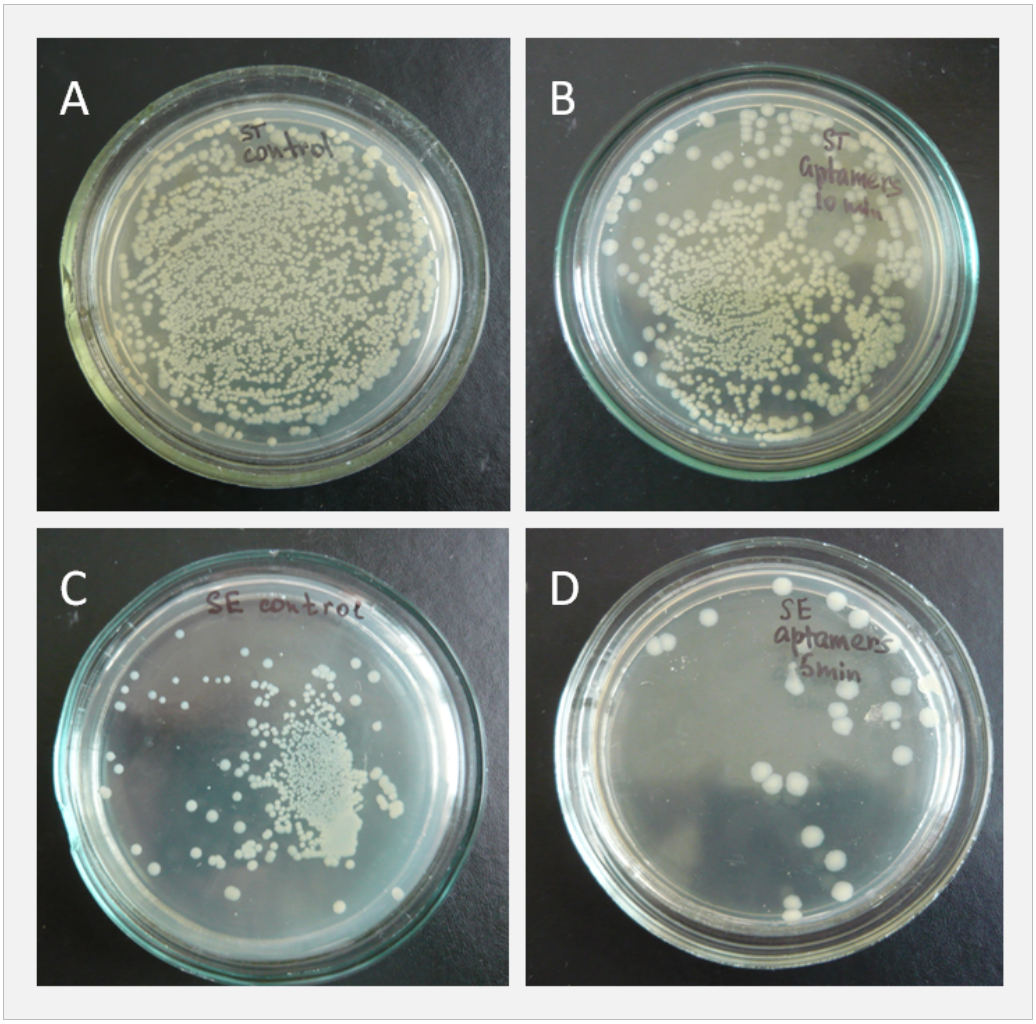


Figure 6. Bacterial Plates for determining the bacterial effect of SE aptamers pool 7 and ST aptamer pool 7 on SE and ST cells. The ST control was done and the amount of colonies were counted (A). THE SE control was done and the amount of colonies were counted (C). The ST aptamers were incubated with ST cells and plated (B). The SE aptamers were incubated with the SE cells and plated (D).

Table 1. Influence of the aptamer pools and clones on bacteria growth of *Salmonella Enteritidis* and *Salmonella Typhimurium*

Number of plates (3 plates) ssDNA concentration 1μM	*Salmonella Typhimurium growth supression % from control sample*	*Number of the colonies Salmonella Enteritidis, % from control sample*
Control (no ssDNA)	0	0
Control (ssDNA library)	9,4	10,1
Clone SE6	42,1	---
Clone SE20	42,8	---
Clone SE22	28,7	---
Clone ST1	---	36,8
Clone ST20	---	12,5
Clone ST12	---	45,6
Pool ST	70,9	23,2
Pool SE	32,1	82,2

The results showed that he combination of clones worked better than individual ones. Also the aptamer pool for SE was able to show 17.8% inhibition of SE growth. The aptamer pool for ST was able to show a suppressed growth of ST (76.9%) (Figure 6). The suppression results were organized in table 1. The control (ssDNA) showed very little growth suppression of SE or ST bacteria.

Future works are required to find the cause of the antibacterial effect. Also the membrane proteins can be analyzed to be used as biomarkers for SE and ST bacteria.

Conclusion

In summary, the work performed in this project has allow the determination of aptamers that are specific to Salmonella Enteritidis and Salmonella Typhimurium bacteria cells. The method used for the selection of aptamers is cell SELEX. This method allow specific aptamer selection of SE and ST. It was also found that the aptamer pools that have the highest affinity (pool 7 for SE and ST) exhibit antibacterial effects. The ST and SE aptamer pools were cloned, sequenced and analyzed. The clones were chemically synthesized and incubated with the SE and ST bacteria cells. The cells were then plated on petri dishes. The antibacterial effect of these aptamer pools and clones are prominent. Further studies will be done to determine the theory behind the antibacterial effect of these surface binding aptamers.

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