Where the wild stuff grows: Bacteria from the cow to the Pasture-izer

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TABLE OF CONTENTS

Page

INTRODUCTION

Bacteria in milk can come from two main sources; Organisms transported from the environment in the milking machine and mastitis organisms from within the udder. Bacteria deposited in the milking and milk-handling equipment multiplies and becomes a major source of contamination if the equipment is not cleaned and sanitized properly.

All farms periodically conduct some form of testing for bacterial contamination to assure compliance with national, state and local milk plant requirements. These tests usually include the Somatic Cell Count (SCC), Standard Plate Count (SPC) and may also include the Preliminary Incubation count (PI) or other test. Using these test we looked at bacterial counts from the time the milk leaves the cow to the time that it enters the High Temperature Short Time pasteurizer. Our aim was to see if there was an increase in counts or where the bacteria were coming from by taking the SCC from the initial cow, comparing it to the counts in the tank

Our goal was to evaluate the cleanliness of the milk when it enters the pasteurizer, and identifying any possible contamination sites along the way.

LITERATURE REVIEW

For a farm to maintain a Grade a certification to ship milk it is important to keep a close eye on the microbial load present in their milk. Bacterial and somatic cell counts (SCC) used as indicators of raw milk quality. The legal limit for Total Bacterial Count (TBC) is less than or equal to 100,000 colony forming units per milliliter (cfu/ml) and Bulk Tank Somatic Cell Counts (BTSCC) cannot be greater than 750,000. This study was conducted in Wisconsin, in California the permutable limit is only 600,000 (Ruegg, 2009). To find the TBC, the most common methods to estimate are; Standard Plate Count (SPC), Petrifilm Aerobic Count (PAC), and Plate loop methods.

In a 2007 study conducted on 268 farms milking roughly 29,000 cows in the Czech Republic, SPC of bulk tank milk samples (BTMS) were the most widely excepted criteria for measuring milk quality no matter of the location of the production. The research stated that the health and hygiene of the cow are very important influencing the level of microbial contamination of raw milk but equally valid is the temperature, length of time in storage, and bacteria present in the bulk raw milk. High bacteria can result of a combination of factors stemming from bulk tank bacteria. To obtain consistent and reputable results, plate agar and incubation for 72 hours at 30 degrees Celsius were used for enumeration of SPC, while MacConkey's agar #3 was used for coliform counts (CC). When inquiring as to a certain bacteria such as P. aeruginosa, plates were incubated at 35 C for 48 hours, while incubating at 37 C for 48 for enumeration.

Looking at the results of the test, it was found that 77.4% of BTMS contained mammary pathogens, while the remainder being free of pathogens, while increasing plate counts in bulk

tank milk can be accompanied by high contamination with coliform bacteria and/or gram negative non-coliform bacteria. It was also found that high counts of mastitis pathogens in herd bulk milk samples were detected in herds with high incidence of S. uberis mastitis. This study showed that the most important contributors to elevated SPCs in BTMS are streptococci and enterococci such as E. faecalis and S. uberis, along with E. faecium and S. dysgalactiae. Along with these, a positive correlation between s. aureus counts and SPC was discovered. An important item to note is that for this study to be universally interpreted, SPCs, CCs, and bacterial counts were adjusted using logarithmic transformation (log base 10)(ACTA VET, 2009).

The bacterial limited growth temperature is <7.2 C (44.95 F), thus is the maximum temperature that a milk truck driver can pick up the milk from a farm. For those not familiar with the temperature sensitivity of the bacteria in milk, for every 0.1 C increases in milk temperature, the odds of increased CC increase by 1%. For every 10,000 cell/ml increase in SCC, the odds of increased CC increase by 4.3%. While the odds of increased TBC are 6.3 times greater for loads with increased CC compared to milk loads with CC less then than the threshold. Ever 10,000 cell/ml increase in bulk milk SCC increased the odds of increased TBC by 2.4%. Another important note is high SCC raw milk has more lipolysis and proteolysis then low SCC raw milk. It also indicates that higher enzymatic activity in high-SCC raw milk affected the quality of pasteurized milk by accelerating the development of sensory defects such as rancidity and bitterness (Ruegg, 2009). This bares great weight when one looks at what happens when lipolysis occurs; it is the breakdown of the fat stored in the fat cells, releasing them into the milk as free fatty acids. Observing the actions that occur following proteolysis, it is seen that is the hydrolysis of proteins into peptides and amino acids cleavage of their peptide

bonds. Following the proceedings of lipolysis and proteolysis there is a greater understanding of why the milk would change in flavor and pH. In the conclusion of the study, there was a small correlation found between SCC and TBC and SCC and CC in the same load (Ruegg, 2009). Variations were also found in the studies with results as such; the variation was least for Lab Pasteurized Counts (LPC), but among farms, LPC had the greatest variation of any milk quality indicator. This suggests that although farms varied in LPC, these values did not vary as much as TBC and CC. This can be attributed to the fact that all the milk was pasteurized prior to LPC tests, thus the pasteurizer did its job of killing off bacteria. Bulk milk loads with increased C were much more likely to have increased TBC compared with loads with CC < 160 cfu/ml (Ruegg, 2009).

A common indicator of the cleanliness of a cow and their environment is by the coliforms found in the milk. In a study done on 60 cows producing an average of 8,894 kg/ cow / year, they were checking for the environmental impact on coliforms. The results were justified as a count of <5,000 CFU/ mL indicates proper hygiene, and a count of < 10,000 CFU/ mL should be achievable by most dairy farms. This study found 50% <5,000, 71% <10,000 (Dingwell, 2009).

To ensure the quality of the food that is being distributed to the consumers, most food microbiology labs are testing for total count and coliforms/ E. coli counts. If the total counts exceed 10 mil CFU/ mL, the food will likely spoil. When testing for fecal contamination and it comes back positive, Escherichia is pink to red colony while other will be blue. PKC4 will give red spots for E. coli, turquoise spots to Streptococcus, blue gray spots for Klebsiella, brown spot for Proteus, cream spot for Pseudomonas and gold spot for staphylococcus aureus. The lactoperoxidase system helps to slow down the growth of some gram negative and gram positive bacteria (Maneerat, 2004).

In another study done on bulk tank milk from a 2 week study on 13 farms, TBC spikes were analyzed. It was found that s. uberis was associated with all five spikes and was the sole organism associated with the first three. E. coli was the second organism associated with the last two larger spikes. Also E. coli had greater magnitude then spikes associated with other organisms. While this study showed the value of selective plating, it is limited due to the specifics of it only being selected spots. From this presentation we know that S. uberis causes inflammation of mastitis and can shed up to 10,000,000 CFU/ mL in their milk (Boor, 2001).

The inflammation of the mammary tissue of the dairy cow is the highest costing disease facing the dairy farmer today. The direct cost of mastitis is 91.00, with 60% of the direct cost as milk dumped with another 205 from actual drug costs. On average subclinical mastitis is estimated at 150.00 dollars/ cow/year. Of all the antibiotic treatments that are administered, 80% are in relation to mastitis. Cure rates for mastitis caused by Staph aureus have been shown to decrease with age (from 81% for cows less than or equal to 48 months of age to 55% for cows greater than or equal to 98 months) (Leslie).

To get a better understanding of what caused mastitis, one must comprehend what is happening inside of the mammary tissue. The primary factor affecting SCCs is infection status. Somatic cells are predominately made if white blood cells (leukocytes) and can be used to gauge the level of infection. When the SCC is 100,000 to 199,999 cells/ml, the presence of infection can be ruled out by bacterial testing. When an infection is present in a mammary gland, milk-secreting cells are damaged and their capacity for producing milk is lowered. Since SCCs are a reflection of infection, the SCC can be used to estimate production losses. A few cows can have a significant impact on bulk tank SCC. For example, assuming the same production level, four cows at 100,000 SCC plus one cow at 1,600,000 SCC would give the same bulk tank average as

five cows at 400,000 SCC, while production losses would be quite different for these two groups. Producers can use SCCs to gauge the level of subclinical infections within the herd and to estimate production and profit losses due to this hidden issue. Attention must also be drawn to the regulations that the farmer must abide by as the "Grade A" Pasteurized Milk Ordinance (2005) states that the SCC for individual producer milk should not exceed 750,000 cells/ml (Cambell).

Digging deeper into what are somatic cells it is seen that more than 95 % of somatic cells are leucocytes, including neutrophils, macrophages and lymphocytes. The major mastitis pathogens Streptococcus uberis, Escherichia coli, Staphylococcus aureus, Streptococcus dysgalactiae, and Streptococcus agalactiae elicit a greater somatic cell response than the minor pathogens Corynebacterium species and Coagulase-negative staphylococci. Although a raised SCC is an accepted indicator of an existing bacterial infection, a very low SCC has been associated with an increased subsequent susceptibility to clinical mastitis. This suggests that somatic cells may provide protection from bacterial colonization as well as being a marker of infection. Neutrophils have non-specific immune function and are important in reducing bacterial numbers early in the course of disease through phagocytosis. During bacterial invasion, there is a rapid and massive influx of neutrophils, to the extent that a transient leucopenia can result. For every 100,000 cells/ml increase in BMSCC there is a 10 % increase in the prevalence of infection in the herd.

It is important to remember that, at the cow level, SCC is already a dilution of the inflammatory process as infection occurs at the quarter level. Factors that influence individual SCC are: bacterial infection- up to 200,000, Stage of lactation- gradually increasing beginning 2 weeks post parturition, time of year- mainly higher in spring and summer when moisture is

increased, time interval- 3x milking yields higher counts then 2x, and measurement error- laboratory handling of samples and accuracy of the automated testing equipment. The California Mastitis Test (CMT) should be used to identify the quarters of those cows that are infected and thus from which quarters milk samples should be taken (Bradley, 2005).

To get a bacterial number to which you can count, one must employ the plating technique. This is when a person makes or uses petri dishes that contain agar. Agar is a polymer made up of subunits of the sugar galactose, and is a component of the cell walls if several species of red algae that are usually harvested in eastern Asia and California. It is also a phycocolloid extract from a group of red-purple marine algae (class Rhodophyceae) including Gelidium, Ptercladia, and Gracilaria. Gelidium is the preferred source of agar. We use agar because, unlike gelatin, won't be degraded (eaten) by bacteria. Also, agar is firmer and stronger then gelatin.

Agar is a gel at room temperature, remaining firm at temperatures as high as 65 C. Agar melts at approximately 86 C, a different temperature form that at which it solidifies, 32-40 C. This property is known as hysteresis. Agar is generally resistant to shear force; however, different agars may have different gel strengths or degrees of stiffness. Agar is typically used in a final concentration of 1-2% for solidifying culture media. Smaller qualities (0.05-0.5%) are used in media for motility studies (0.5% w/v) and for growth of anaerobes (0.1%) and microaerophiles.

Specifications for bacterial grade agar include good clarity, controlled gelation temperature, controlled melting temperature, good diffusion characteristics, absence of toxic bacterial inhibitors and relative absence of metabolically useful minerals and compounds. The

types of agar that one might use as bases for grown would be: Blood agar- made of blood cells and grows most bacteria, LB (Luria Bertani) Agar- a subtype of nutrient agar, is a general medium, and does not prefer one bacterium over another, MacConkey Agar- grows only gram-negative bacteria, E.coli grow into red colonies, two types: one with lactose sugar and one without added sugars, Non-nutrient agar- usually not suitable for growing bacteria, but may be used to grow other microorganisms, Nutrient agar- grows the largest number of different types of microbes- fungi and bacteria. Sometimes is to rich to grow all, Tryptic Soy agar- basic medium, mainly used as an initial growth medium for the purpose of: observing colony morphology, developing a pure culture, and culture storage.

Although pre-poured agar plates are available, one can make agar plates tablet, powered, or bottled agar by following a few simple instructions. The formulation for LB agar is: 9.1 g/l tryptone, 4.6 g/l yeast extract, 4.6 g/l NaCl, and 13.7 g/l agar. 500 ml of agar will pour about 25 (100 mm diameter) Petri dishes. The ideal temperature of incubation is 32 C or 90 F. Incubate upside-down (Space Buddies, 2009).

Incubation is a vital part of obtaining growth that is countable and is proportional to the organisms contained within the sample. To get to the point of a countable number, dilutions will be necessary. Detection and enumeration of specific organisms or groups of organisms are performed by serial dilutions of milk samples (Boor, 2001). Once plated on selected media, the plates or films must be placed into an incubator for the instructed time. For CC, the incubation time is 24 hours at 32 C and 48 hours at 32 C for SPC (Costello, 2001).

MATERIALS AND METHODOS

Making Agar:

Tryptic Soy Agar (TSA):

TSA agar is made by mixing 20 grams of Tryptic Soy Agar and 1 gram of soluble starch with 500 mL of distilled water.

1. Place water in clear glass beaker.
2. Set on Hot plate with magnetic stir bar.
3. Start sir at 250 rpm and heat at 320 Degree Celsius.
4. Add TSA and starch slowly.
5. Continue stirring and heating until mixture becomes non-opaque and begins to boil. Do not allow to boil for greater to than 1 minute as it may denature the nutrients.
6. Turn off stirring and heating, remove from hot plate using rubber mitts.
7. Pour into 5-150 ml autoclaveable glass containers.
8. Insert into autoclave for sterilization, run for 15 minutes at 121 C.
9. Remove and pour 15 mL into each Petri dish.

Standard Plate Count Agar (SPC):

SPC agar is made by mixing 11.75 of Standard Method Agar with 500 mL of distilled water.

1. Place water in clear glass beaker.
2. Set on Hot plate with magnetic stir bar.
3. Start sir at 250 rpm and heat at 320 Degree Celsius.
4. Add TSA and starch slowly.

5. Continue stirring and heating until mixture becomes non-opaque and begins to boil. Do not allow to boil for greater to than 1 minute as it may denature the nutrients.
6. Turn off stirring and heating, remove from hotplate using rubber mitts
7. Pour into 5-150 ml autoclaveable glass containers.
8. Insert into autoclave for sterilization, run for 15 minutes at 121 C.
9. Remove and pour 15 mL into each Petri dish.

Petrifilm E. Coli/ Coliform Count Plate

Petrifilm E. Coli/ Coliform Count Plates contain Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, and an indicator that facilitates colony enumeration. Most E. coli (about 97%) produce beta-glucuronidase, which produces a blue precipitate, associated with the colony. The top film traps gas produced by the lactose fermenting coliforms and E. coli. About 95% of E. coli produce gas, indicated by blue to red-blue colonies associated with entrapped gas on the Petrifilm EC plate.

Pouring plates:

Pour approximately 15 mL into each Petri dish, making sure it covers the bottom of the dish. Allow to dry for 15 minutes and place in refrigerator until ready to use.

Swabbing:

1. Label the swab.
2. Bend the red snap valve at a 45-degree angle until you hear the valve break. This allows the letheen broth to flow into the tube and wet the swab.

3. Squeeze the bulb of the swab to transfer all of the letheen broth to the tube end of the swab.
4. Twist and pull apart the bulb end of the swab from the tube end of the swab, which contains the letheen broth.
5. Hold the swab handle to make a 30-degree angle with the surface. Rub the swab slowly and thoroughly over the desired surface area. Rub the swab three times over this surface, reversing direction between alternating strokes.
6. After sampling in complete, securely insert the swab back into the swab tube and transport to the lab for inoculation. Plate letheen swab solution as soon as possible.
7. In the lab, vigorously shake or vortex the swab for 10 seconds, to release bacteria from the swab tip.
8. Wring out the contents of the swab tip by pressing and twisting the swab against the wall of the tube.
9. Carefully pour the entire contents of the tube onto a dilution.

Swab Collection Sites:

Tank 2, front agitator: The agitator is the paddle that stirs the milk and is responsible for equal cooling throughout the product. On the agitator, along both sides of the shaft (2 inch by 1 inch rectangle) and the undersides of each paddle (2 inch by 2 inch square). Each sample taken was 3 collections of the shaft, 3 collections of the paddle in alternative patterns.

Receiver Jar, Left side of parlor: The receiver jar functions as the collection point of the milk from the top and bottom milk lines of the machines. It acts as a balance tank in that when a designated level is reached inside of the tank, a circuit is completed using metal probes and the milk is pumped to the bulk milk tanks in another room. This is the vital connection between the

cows and the raw tanks, with all milk coming into contact with this piece of equipment prior to storage. Swabbing on this piece was conducted on a 2 inch by 2 inch square from the middle of the 7 inch top plastic part.

Overhead of tank 2: Overhead of tank 2 is the end of the pipe that goes from the milk plate heat exchanger to the "L" joint that puts the milk into the tank. This is the side of possible residual milk buildup while milking or post-milking if the connections are not correctly fitted. The swabbing took place on the end and into the pipe approximately 1 inch. Note should be taken on the gradual increase in size of the end of the pipe prior to the end, starting 2 inches from the end. This leads to moisture retention after all cleaning has been completed, or if cleaning is not performed properly, a microbial kingdom.

Milk Truck for Creamery: Taking into account the age of the milk truck that is used to transfer the milk from the raw milk tanks on the farm to the storage silos at the plant, sanitation of the truck and keeping a close eye on the bacterial counts that are resulting post-hauling are pertinent. Each of the times that swabs were taken sanitizer water residue was in the bottom of the tank. For this reason all samples were taken 12 inches in from the left side, with the sample area being roughly a 3 inch circle. Note should be taken that there was a lid fitted with a filter to allow for ventilation.

Back of Tank 2: Looking for sites that are least likely to be properly cleaned led to the swabbing of the back of tank 2. When a tank is washed, water is pumped at a certain rate up a pipe and pushed through a spray ball that distributes the water and additives to all parts of the tank. Questions are raised at what pressure this should be and for how long would it need to be for all parts to be cleaned sufficiently. When taking this swab of 2 inch by 4 inch, note was

taken that there was water residue still on the tank. The residual would imply that there was cleaning, but does not guarantee that it killed or cleaned all portions of what could grow. The collection was done in the crease of the rear right corner.

Bottom of Tank 1: Being the only place that the milk comes into contact with that is not CIPd, the bottom of tank 1 was pertinent sample this site, because it is here that the milk weight is taken twice daily, as well as where all the milk passed prior to being loaded onto the truck. Upon initial observation, the sites looked clean to the naked eye and bare no odor. The swabbing was conducted on a strip 1.5 inches wide by 2.5 inches long, stating at the beginning of the curve and ending at the last spot able to reach with the swab going in from the bottom (right) of the steel rod. Notation should be taken that when asking the herd manager, this site is regularly rinsed with hot water after each milk reading is taken and a cap is screwed on to keep outside contaminants out.

Dilutions:

Dilutions are useful in trying to obtain a visual count of the bacteria present in a select medium.

1. Collect representative sample using above swabbing technique. This will yield 1 ml of letheen broth.
2. Pour broth from 3M quick swab into a 99 ml dilution blank. Rotate 20-25 times to ensure equal distribution of broth in solution. Your yield will be 1:100.
3. Using a sterile tip and pipette, pull 1 ml of 1:100 solution. Deposit into a 9 ml dilution blank, thus yielding 1:1000.
4. Repeat with 9 ml blanks to desired dilution level.

Spreading to Counting plates

There are two ways of plating dilutions, pour plates, or spread plates. In this research, we will be using spread plates due to easy and being able to refrigerate of unused plates for future use.

When we are counting plates, it is important to note the dilution that took place prior so to ensure that correct results are recorded. Records of 25-250 will be written for ease of counting with greater than 250 being said to be to numerous to count (TNTC).

In the scope of the project, we will also be using Petrifilm to count coliforms. These are premade plates that are a sandwich style media that will show color as well as gas production.

Spread plate:

1. On the bottom (part that contains the agar), write initials, agar type, date plating, were from, and dilution.
2. Light Bunsen burner to create a sterile environment. All plating will be done within 12 inches of flame.
3. From the selected dilutions, using a sterile tip for each new intensity, obtain 1 ml of sample using a pipette.
4. Carefully open the top of the plate to 30 degrees, paying close attention to prevent exterior contamination.
5. Squirt 1 ml into center of media.

6. In a spare sterile Petri dish, fill one-third with 90% ethanol. This will be used to sterilize the spread tool between each plate spread.
7. With a sterile spreader, in a circular motion, complete disperse sample over entire surface.
8. Replace lid and allow to set for 15-20 minutes undisturbed.
9. Repeat for each dilution.

Petrifilm

1. On the bottom of the white cover write initials, agar type, date plating, were from, and dilution.
2. Light Bunsen burner to create a sterile environment. All plating will be done within 12 inches of flame.
3. From the selected dilutions, using a sterile tip for each new intensity, obtain 1 ml of sample using a pipette.
4. Carefully lift the parchment-like paper 45 degrees, paying close attention to prevent exterior contamination.
5. Squirt 1 ml into center of media.
6. Slowly lower cover to prevent air bubbles.
7. Implementing use of the 3M Petrifilm framer, press firmly to equally distribute sample on media.

Incubation:

For SPC and TSA plates, they will be incubated for 24 hours, checked for growth, returned to incubator and checked again at 48 hours. The Temperature for optimal growth is 32 degrees Celsius.

For Petrifilm, they will be incubated for 24 hours at 37 degrees Celsius.

Counting:

1. Wearing gloves, remove plates/ Petrifilm from incubator.
2. Enlisting the aid of the plate counter and a fine tipped black sharpie, count each individual colony. Precaution should be taken not to inhale any vapors from within the dishes, as they may be detrimental to your health.
3. Record all information on the bottom of sample as well as the count
4. Perform calculations for dilutions.

RESULTS

The results of my first trial were completely contaminated. Hypothesizing on why there was total contamination on all plates yielded a few possible explanations; there were staph aurus on hands, spores floating in the air, contamination post-swab, agar contaminated, or no aseptic environment. Disproving each of these are as follows: we were wearing gloves while swabbing and plating, so no contamination could have occurred. The agar that was made was autoclaved prior to pouring into Petri dishes, so any foreign materials that may have accumulated inside of the agar storage container would have been killed. Aseptic environment was achieved during all plating times, disproving the previous hypothesis. The conclusion that was reached as to possible validation for contamination is either spores that were floating in the air while pouring or that there was post-swabbing contagion. As a preventative measure for future testing, I kept all swabs in the plastic bag, thus attempting to attain results on future. In the remaining two trials, there were reputable results that were significant in developing a base to compare in the future.

Results for the front agitator inside of tank were consistent throughout showing very little growth. TSA was a better growth media, showing results at 48 hours both times. Reasoning for any growth was attributed to difficult places for the cleaning agent to hit from a spray ball applicator. To fix this problem and achieve lower bacterial counts would be to adjust the agitator speed while cleaning.

After swabbing and plating, growth was not evident in the second trial, yet was highly contaminated in the third. Upon further inquiry into why there was such high growth in the third trial, I was told that the wash had not been properly that morning. This is a very good comparison of what happens if not all protocols are followed or if someone forgets to change

valves on the cleaning line. Solutions for this growth on the third is to make sure that all employees are well versed in the importance of making sure all pathways are open when running the wash.

Minimal growth was discover on the pipe over tank two, with slight variation from trial two to trial three with trial three showing higher counts. The colonies that did grow looked to be from the rim of the pipe where it connects to the elbow, a rough surface. The increase shown in trial three follows the pattern of the receiver jar in that the wash had not been properly. Along with making sure that the wash is run correctly every time, a gasket may be beneficial to the operations sanitation.

One colony was found on the plate from the milk truck that the creamery operates as transportation. With reference to the very low count that grew, observations were done on the possible places that could be improved to gain complete sterility. Conclusion was that the place in which a black mold-like growth would occur would be from the exterior air, thus by regularly changing the vent sock would solve this problem.

The results from the testing done on the back of tank two were inconclusive. There was not a consistent growth or lack of growth from plate to plate, leading one to believe that there was contamination at some point in the collection or plating process. Further testing would have to be done on this site for precise results to occur. Possible conclusions would be to check that the water pressure going into the tank was great enough to reach the back of the tank and be potent enough to do a thorough job of cleaning.

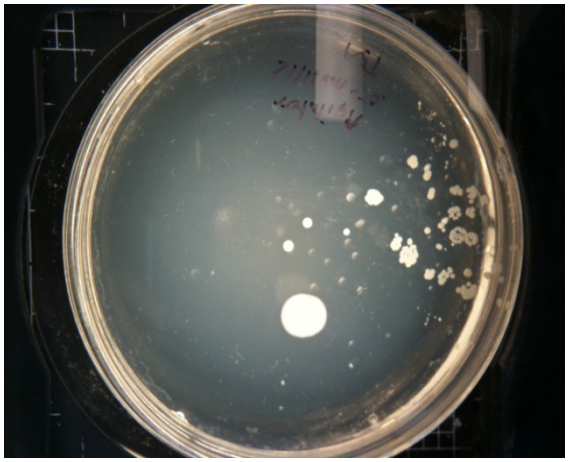
Of the six sites that were swabbed and plated, the bottom of tank one was be far the worst. In trial two it can be seen that there was TNTC on all plates at 24 hours and 48 hours.

Attempts were made to dilute the sample out further with hope of attaining a recordable number, with the results being smaller yet still unable to be counted under 250 cfu/ml. The projected dilution factor would be greater than 10-7, given that there is no outside contamination. Questioning why this site would contain such high counts can be justified when one notes that this is not a CIP site. This particular spot is rinsed with hot water after taking each milk sample, yet is never cleaned with sanitizer. Taking the swab from tank one was pertinent because it is the tank that most commonly contains milk, as this is the first tank to have milk placed in it after the milk truck driver comes. This is important to know since there is no time for it to be place in the cleaning sink in between times it has milk in it. The proposed solution would be to have the milkers rinse the bottom of the tank with a chlorine solution after taking each milk weight. This would kill most of the bacteria present as well as become a preventative measure towards being a basis for future host sites.

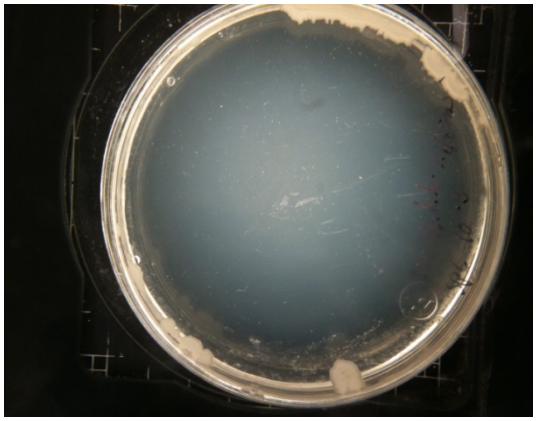
In conclusion, proper sanitation can be the answer to a lot of the bacterial growth that was found through the plating. Educating the milkers on the techniques that will lead to reduced counts, which in turn will yield a higher quality milk. Attention should be placed equally on the details of collecting samples and plating techniques as contamination could yield false positives. Details are the controlling factor when testing for contamination.

In the below sample we see the various results that were yeilded in the project.

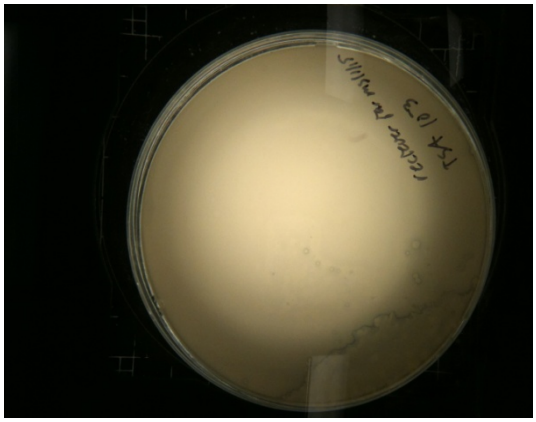
Example 1: In this plate we see the type of results that we are trying to attain, it is under 250 CFU/mL and easily countable. The colonies are well defined, with the remainder of the plate being completely clear.



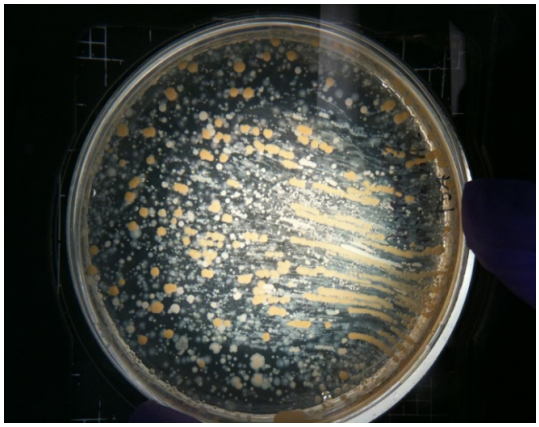
Example 2: Here we see the result of what looks like improper technique, with all the colonies being on the outside of the plate. This is a result of not flipping the plate fast enough, thus all the sample running to the outer edge. It was later learned that the media is higher in the middle then the edges , which causes this result. Numbers on this would be To Numerous To Count (TNTC).



Example 3: With the inability to see any separation between colonies contained within this plate is a strong indication of complete contamination. Seen in this plate is what would be called foreign contamination, something that was not in the swab is on this plate. This plate cannot be counted toward any results.



Example 4: In this example we can see separate colonies, yet it is TNTC. In this case one would want to make a greater dilution to attain more precise results. What can be learned from this plate is that there is growth in that area that it was taken from, and from the uniform color we can say that it is all the same type of growth.



CONCLUSION

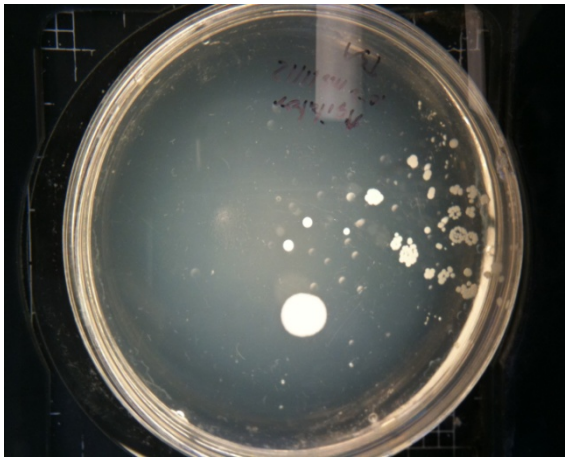
The validity of a project is not based solely on the results, but also by what one might find along the way. The results that were compiled throughout this project varied greatly, yet bared a common theme, if the Clean In Place (CIP) works there is no growth. It is in places that are not CIP, yet come into contact with the milk that are an issue.

The second conclusion to this project is the importance of making sure that all cleaning components are carried out properly and to their fullest. Employee training and reliance of the pertinence of how the CIP is carried out are one of the most beneficial controlling factors in bacterial growth and contamination in the raw milk.

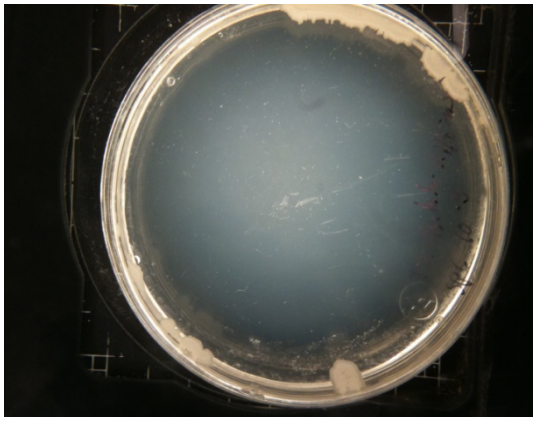
APPENDIX I

Tank 2 Front Agitator

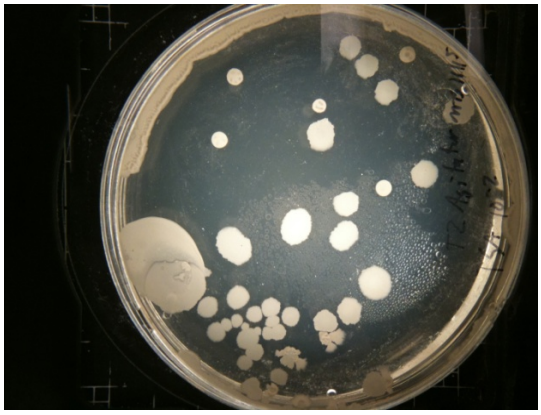
11-15-2009 10-2 TSA



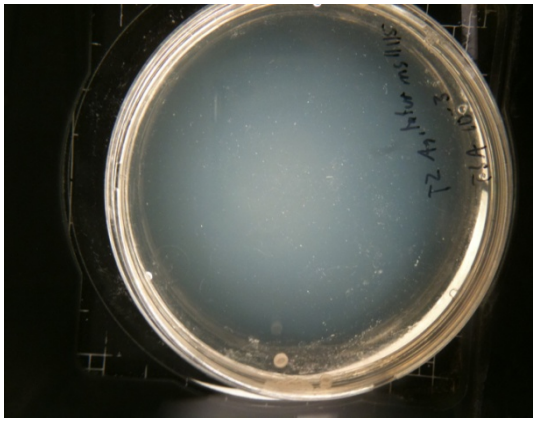
11-17-2009 10-3 SPC



10-2 TSA



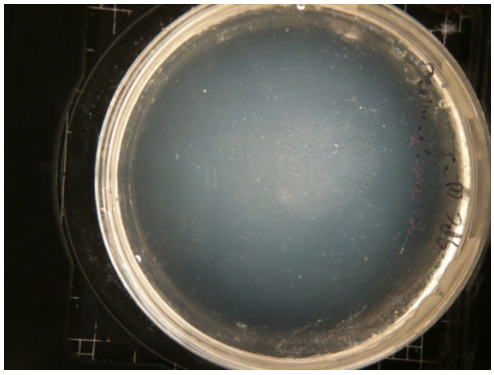
10-3 TSA



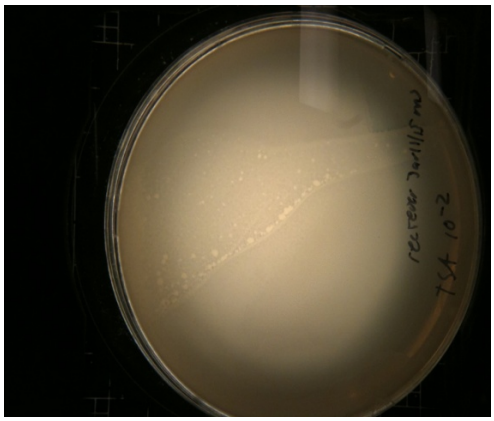
APPENDIX II

Receiver Jar, Left Side

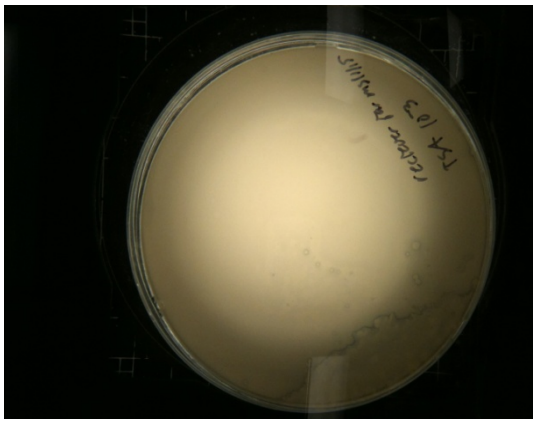
11-17-2009 10-3 SPC



10-2 TSA



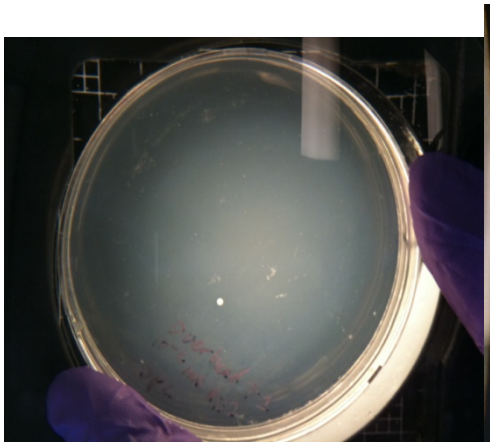
10-3 TSA

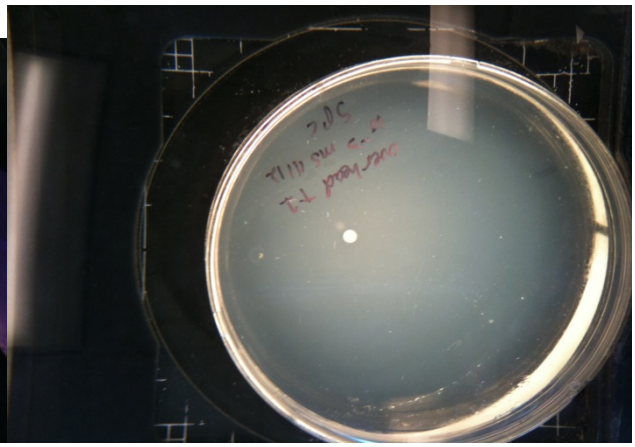


APPENDIX III

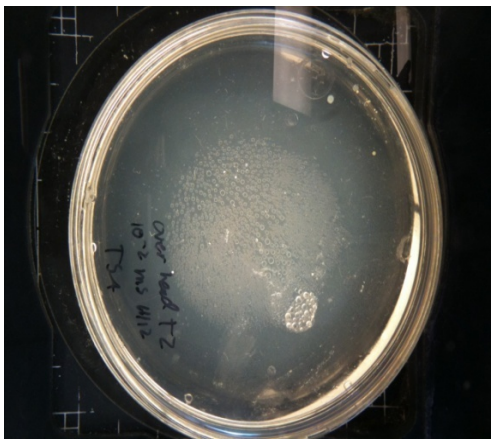
Overhead of Tank 2

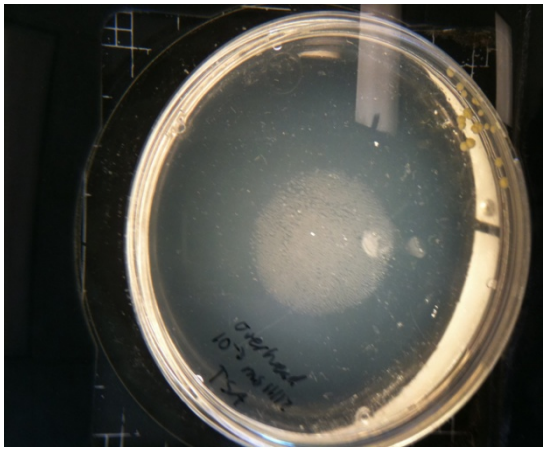
11-15-2009 10-2 SPC 10-3 SPC



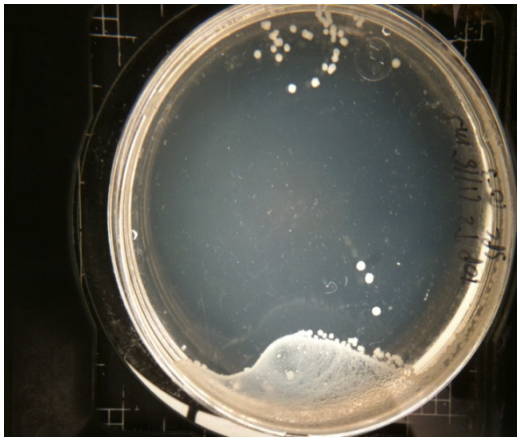


10-2 TSA 10-3 TSA

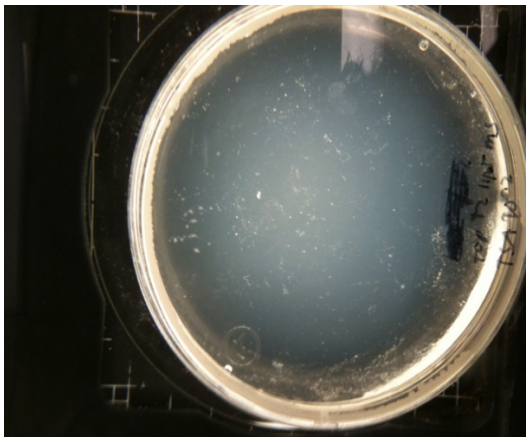




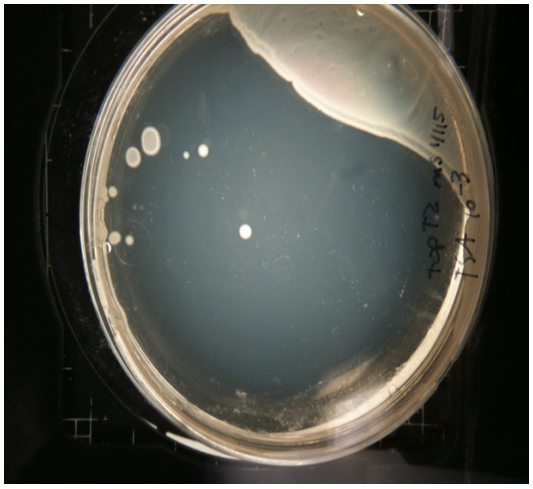
11-17-2009 10-3 SPC



10-2 TSA

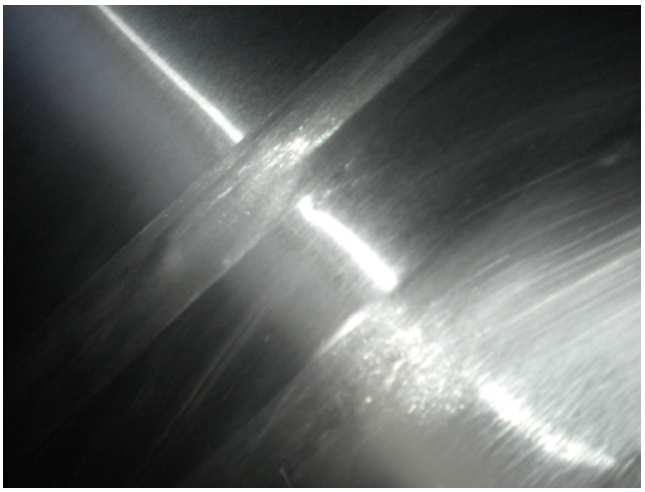


10-3 TSA

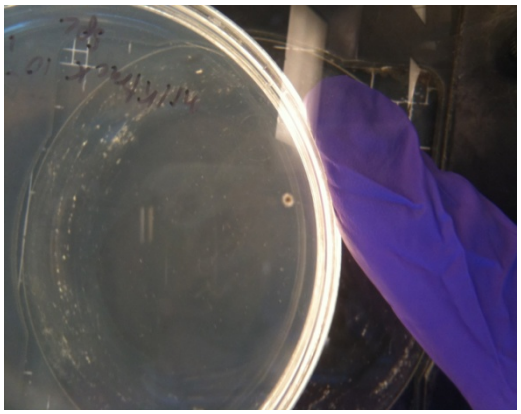


APPENDIX IV

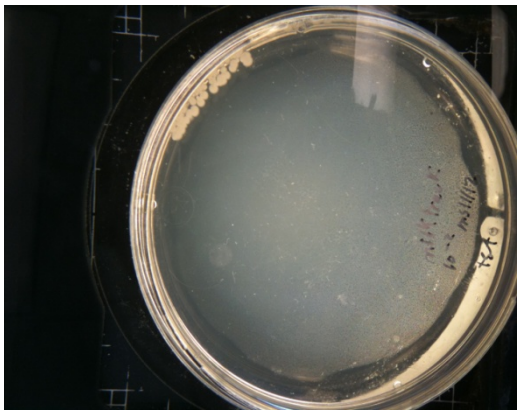
Milk Truck for Creamery



11-15-2009 10-3 SPC



10-2 TSA



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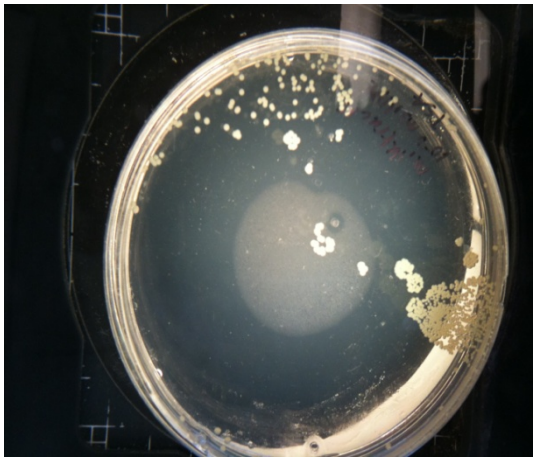
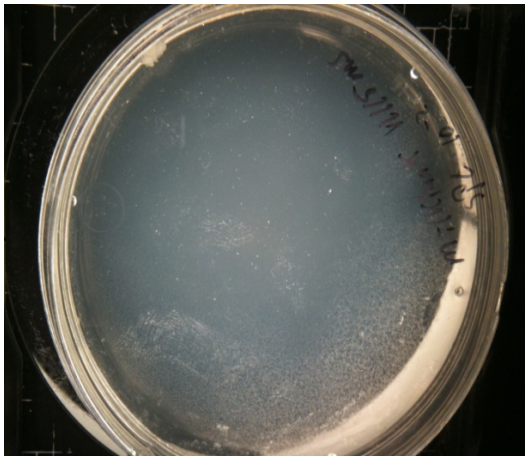


11-17-2009 10-2 SPC



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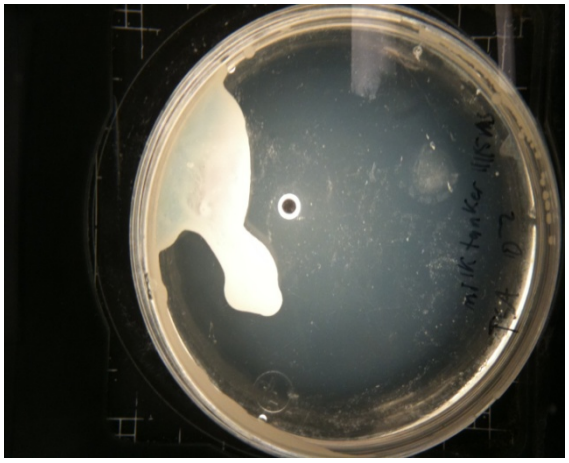
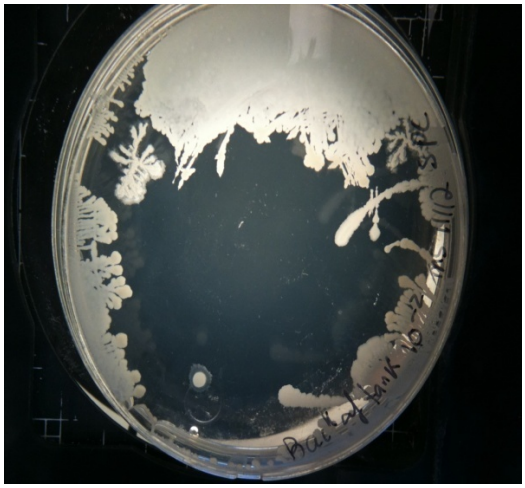


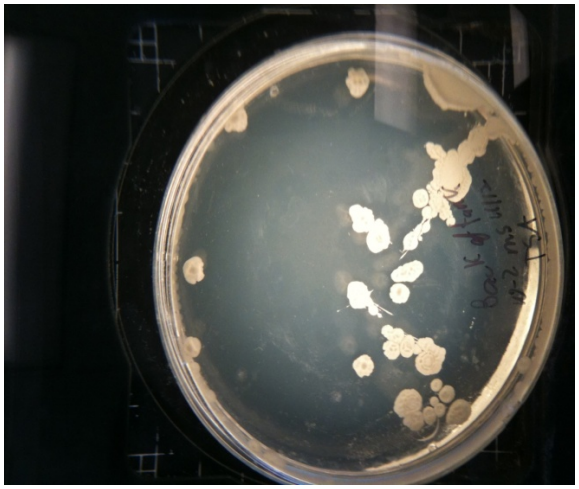
APPENDIX V

Back of Tank 2

11-15-2009 10-2 SPC

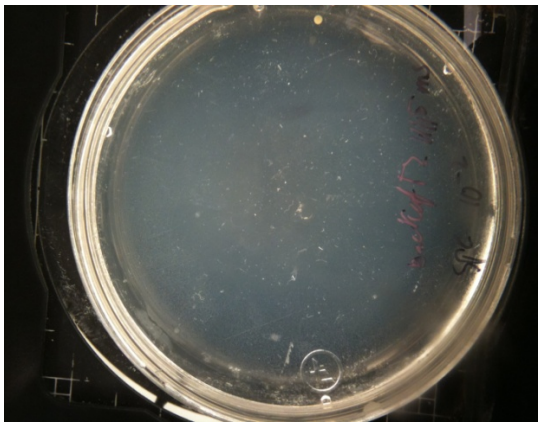


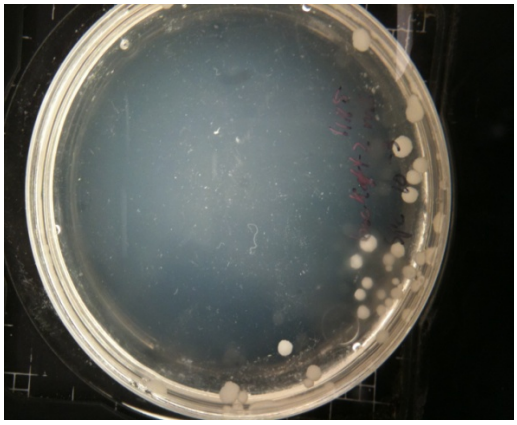
10-2 TSA



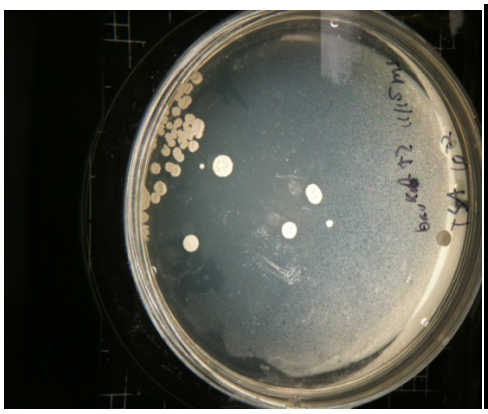
10-3 TSA

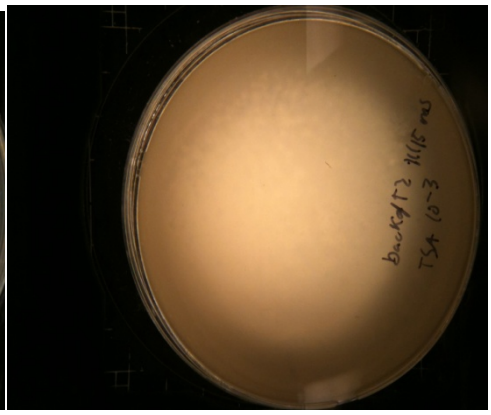
11-17-2009 10-2 SPC 10-3 SPC





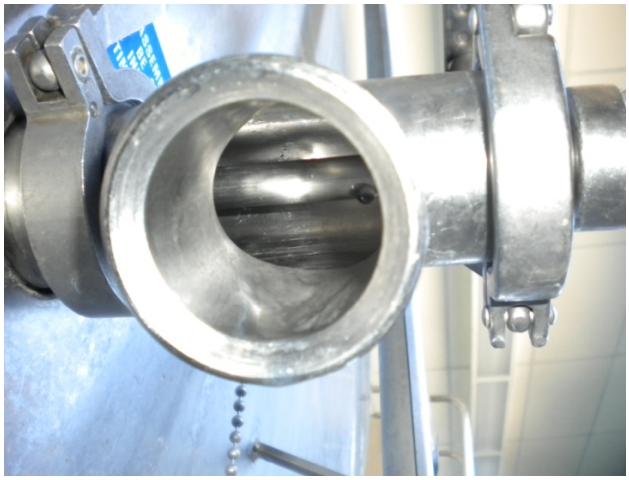
10-2 TSA 10-3 TSA



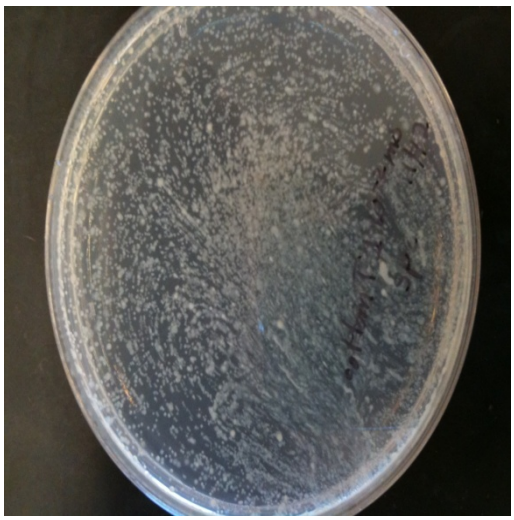


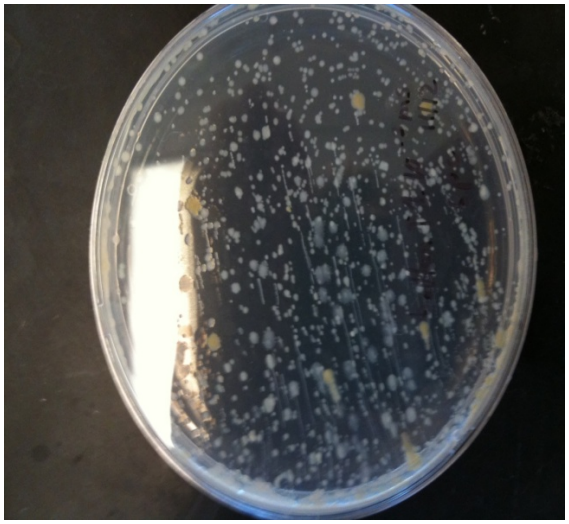
APPENDIX VI

Bottom of Tank 1

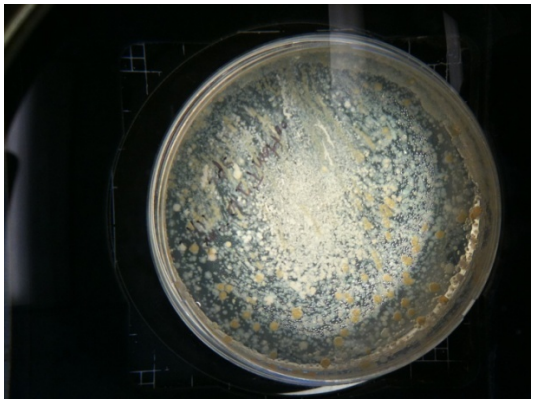


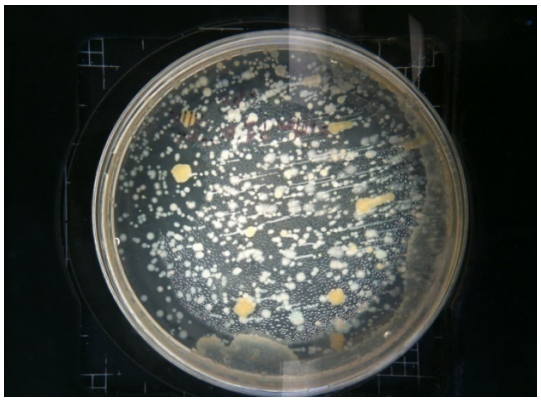
11-13-2009 10-2 SPC 10-3 SPC



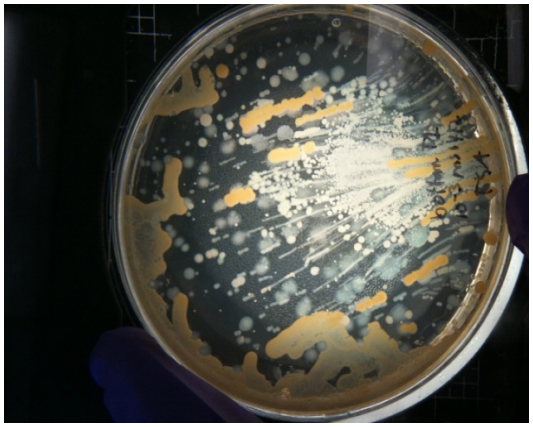


11-15-2009 10-2 SPC 10-3 SPC

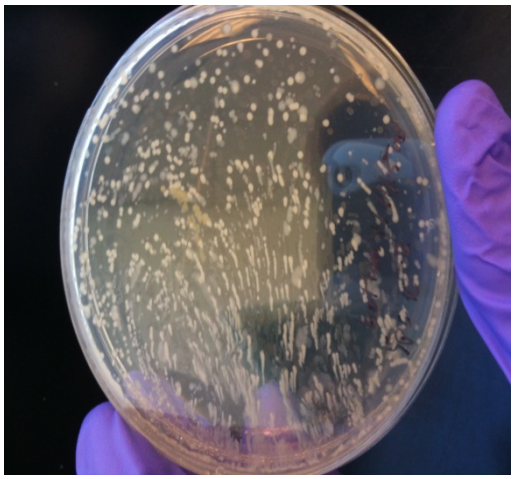




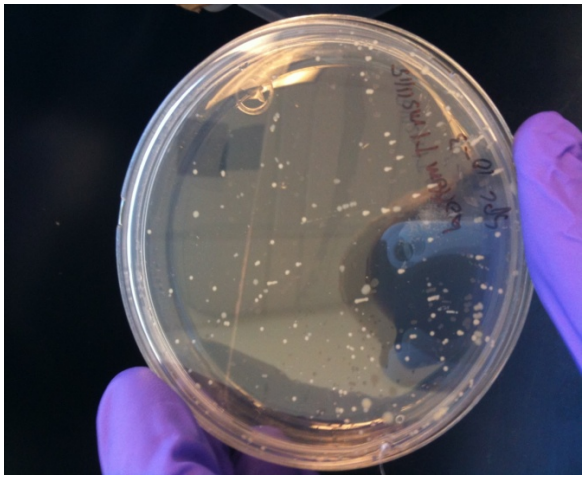
10-3 TSA



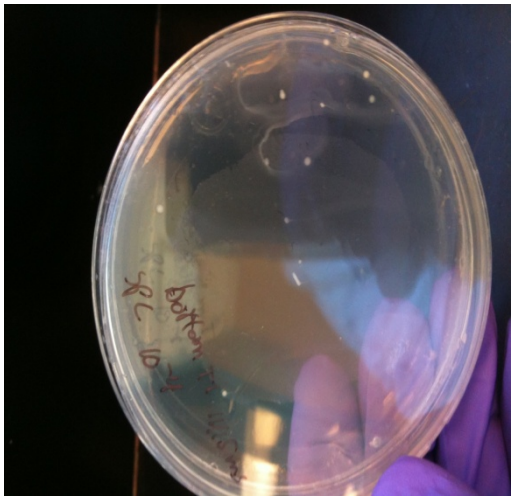
11-16-2009 10-2 SPC



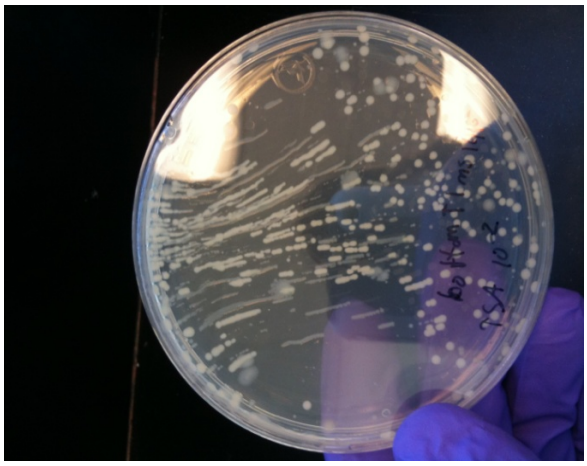
10-3 SPC



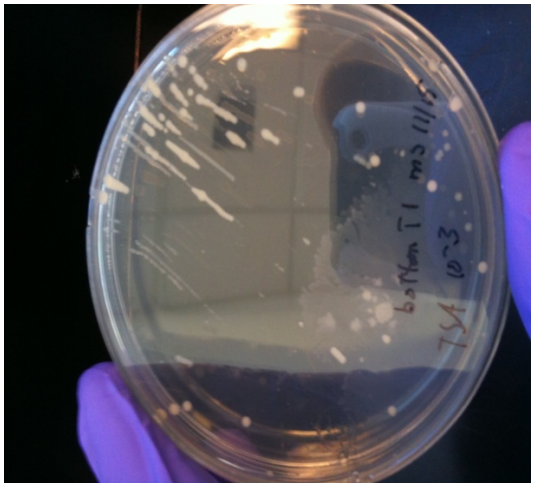
10-4 SPC



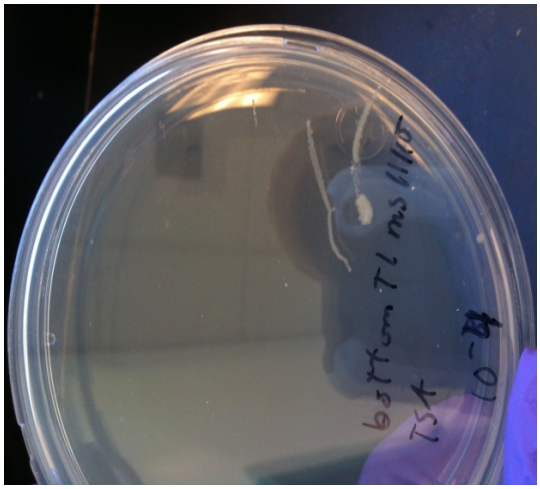
10-2 TSA



10-3 TSA

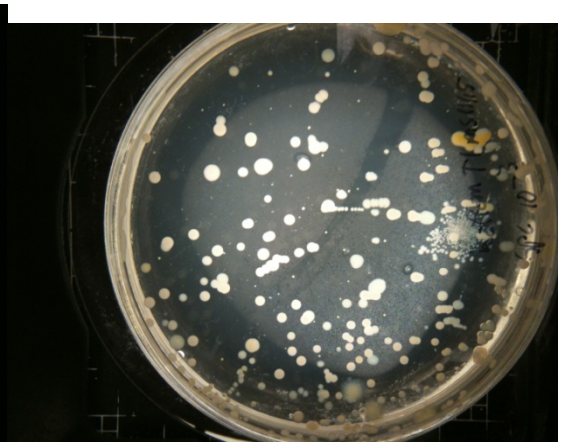


10-4 TSA

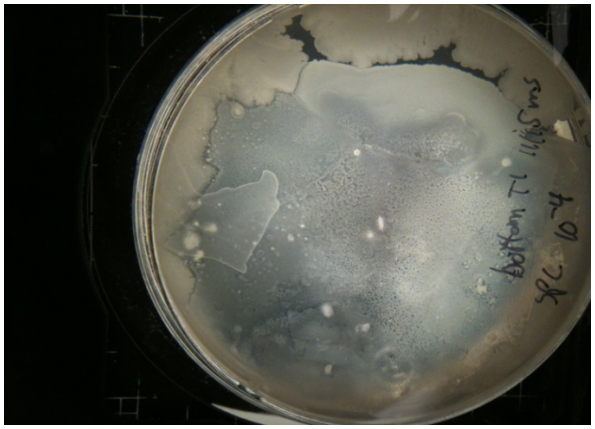


10-17-2009 10-2 SPC

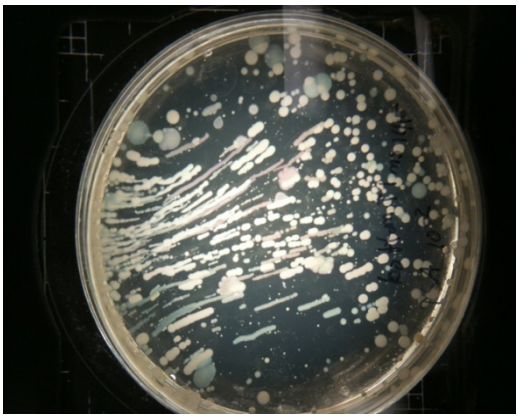
10-3 SPC



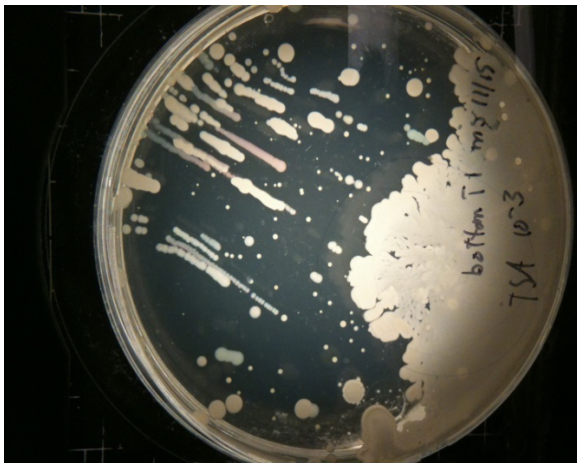
10-4 SPC



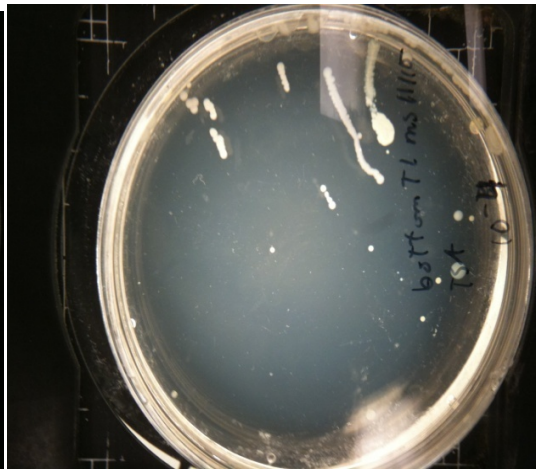
10-2 TSA



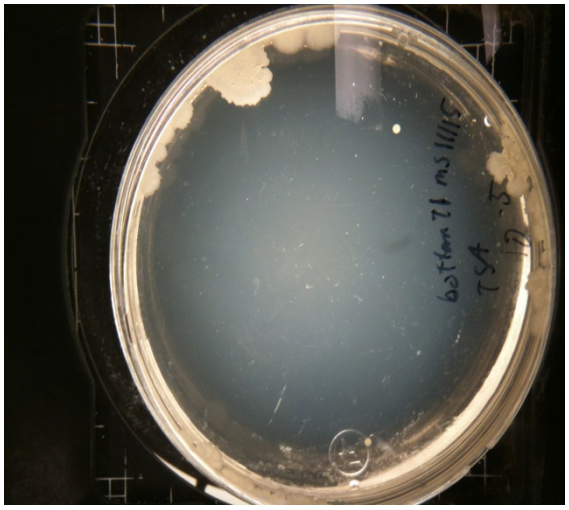
10-3 TSA



10-4 TSA



10-5 TSA



10-2 E. Coli/Coliform

10-3 E. Coli/ Coliform