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Effect of Long Pasteurization Run Times on Bacterial Numbers in Milk

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EFFECT OF LONG PASTEURIZATION RUN TIMES ON BACTERIAL NUMBERS IN MILK

by

Brynli Tattersall

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of

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ABSTRACT

Effect of long pasteurization run times on bacterial numbers in milk

by

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Utah State University, 2020

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Raw milk requires pasteurization to kill pathogens and reduce spoilage organisms before being used in product manufacture. High-temperature short-time (**HTST**) pasteurization is the most common form of pasteurization in the dairy industry. High-temperature short-time pasteurizing is the heating of milk to 72°C for 15 s. The high temperatures in the heating section of the pasteurizer are too high for bacteria to adhere to and grow on the heat exchanger plates, but a few thermoduric bacteria and bacterial spores survive. Once through the heating section of the pasteurizer, these organisms can attach to the walls of the downstream sections of the pasteurizer, beginning the formation of a biofilm. During long run periods, some portions of the downstream regeneration and cooling sections will remain at a temperature permitting spore germination and bacterial growth. Following the attachment of cells, these cells divide and produce exopolysaccharides resulting in biofilm growth and thickening. Cells from the biofilm can then be shed into the pasteurized product. Long pasteurizer operation times are preferred in industry because it requires less cleaning and shutdowns so more product can

be processed and thereby increasing yield. However, these long operation times can result in biofilm-bound spoilage bacteria being released into the pasteurized product with detrimental effects on product quality. As bacterial numbers in the biofilm increase, more bacteria and biofilm material can be released into the pasteurized milk.

Microorganisms released from biofilms can cause defects in milk powders or cheese and also increase its microbial load thus decreasing its value. The presence of bacterial spores is a concern for milk powders because spores can survive in extreme conditions. Once the milk powders are rehydrated, the spores can then germinate and cause spoilage. Both *Geobacillus stearothermophilus* and *Anoxybacillus flavithermus* are thermophilic spore-forming bacteria commonly found as contaminants in milk powders. Nonstarter lactic acid bacteria are also of importance as they are known to cause some defects in cheese. *Streptococcus thermophilus* is a common bacterium known to survive the pasteurization process and cause defects in cheese. *Paucilactobacillus wasatchensis* is a nonstarter lactic acid bacterium that has been found to cause a gassy defect in aged cheddar cheese.

To determine the extent of biofilm build-up and release of bacterial cells into the product over long operation times of an HTST pasteurizing system, a lab-scale pasteurizer was fabricated and operated continuously for 18 h. Samples were collected, and bacterial load determined as a function of time. Bacteria present in each sample were isolated and identified using 16S rRNA amplicon data to determine the constituent species of any biofilm material present in the samples.

(91 pages)

PUBLIC ABSTRACT

Effect of Long Pasteurization Run Times on Bacterial Numbers in Milk

Brynli Tattersall

This project was funded by the Western Dairy Center to understand how long a milk pasteurizer can be operated before increases in bacterial numbers are observed in the pasteurized milk. While pasteurization kills pathogenic bacteria there are some non-pathogenic bacteria that can survive and have the ability to become attached to the surfaces in the cooling sections of the pasteurizer. Some bacteria can also produce spores that survive pasteurization even if the bacterial cells are killed. Temperatures in the cooling section remain in a range suitable for growth of these heat-tolerant bacteria and can allow germination of bacterial spores. While this is not a health issue, it can affect the quality of the milk and other dairy foods if spoilage bacterial numbers become high.

We constructed a laboratory-scale heat exchanger for pasteurizing milk and monitored the number and type of bacteria contained in the milk. The system was operated for 18 hours with a continuous flow of milk being heated (to 72°C (161°F) for 15 seconds) followed by cooling. Sample of milk were collected every hour and then analysed for the number of bacteria and the number of bacterial spores.

Bacteria that would have survived pasteurization (thermophilic bacteria) of the milk stayed at the baseline level for the first 7 hours of processing. There was a 10 to 20 fold higher level of bacteria in the milk after 8 hours processing, followed by another 10 fold increase after 14 hours of processing. Operating a pasteurizer for extended times will lead to increased bacterial load in pasteurized milk which can cause quality problems.

ACKNOWLEDGMENTS

I would like to thank Dr. Donald J. McMahon for his kindness and invaluable support and guidance he provided for me throughout my time as his student. I would also like to thank Dr. Craig J. Oberg and Dr. Almut H. Vollmer for their mentorship. I am thankful for the BUILD Dairy program that has funded my research and offered me many opportunities to grow both academically and personally. I want to thank the Aggie Creamery and its production staff, especially Dave Campbell, as they were a huge help to me during my research. I would like to thank my husband, Dayne Tattersall, for his unwavering support.

Brynli J. Tattersall

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LIST OF ABBREVIATIONS

BLAST – Basic Local Alignment Search Tool

CFU – Colony-Forming Unit

EPS – Exopolysaccharides

HTST – High-Temperature Short-Time

LAB – Lactic Acid Bacteria

MALDI-TOF – Matrix-Assisted Laser Desorption/Ionization Time of Flight

NCBI – National Center for Biotechnology Information

NSLAB – Non-Starter Lactic Acid Bacteria

SE – Standard Error

INTRODUCTION

Pasteurization is one of the most important unit operations in the dairy industry. This process treats milk with heat to kill many spoilage and all pathogenic organisms. Prior to the development of pasteurization, consumption of raw milk lead to the spread of numerous diseases such as tuberculosis, Q fever, and diphtheria (Holsinger et al., 1997). Not only does pasteurization kill human pathogens, but it also increases shelf life. Pasteurization is an important step in many processes including the processing of fluid milk, cheesemaking, and the manufacture of milk powders.

The heating process of high temperature short time (**HTST**) pasteurization is usually performed in a heat exchanger. Two types of heat exchangers are commonly used for the pasteurization of milk (either tubular plate heat exchangers or plate heat exchangers). During the pasteurization process, heat exchangers are used to heat the milk, transferring the heat from the heating medium (usually water) to the cold milk. The heating medium flows countercurrent to the product to minimize the temperature differential between the product and the medium. A high temperature differential can result in fouling (Bylund, 2003).

Pasteurization is not a sterilization process, but a process meant only to kill pathogenic bacteria and reduce spoilage bacteria. It has no effect on bacterial endospores which are extremely heat resistant. While pasteurization is necessary to kill pathogenic bacteria, it is common for other bacteria to survive. There are certain groups of bacteria that can survive pasteurization including thermophilic, thermoduric, and spore-forming bacteria. Though these organisms cannot grow at pasteurization temperatures, they can withstand and survive these high temperatures.

In the dairy manufacturing industry, the processing equipment is required to be cleaned at least once in 24 h. In an effort to maximize profit, the equipment is run continuously with minimal cleaning breaks. Without the mandatory cleaning step however, biofilms have the opportunity to build up on processing equipment and eventually causing reduced processing efficiency, cleaning difficulties, and product contamination (Flint et al., 1997).

HYPOTHESIS AND OBJECTIVES

Hypothesis:

Continuous operation of a heat-exchange pasteurizer over long periods of time causes an increase in bacterial load in heat-treated milk.

Objectives:

1. Design and assemble a lab-scale plate heat exchanger system that allows raw milk to be processed for 18 h at pasteurization conditions.
2. Enumerate bacterial load of pasteurized product over time.
3. Identify select colonies of bacteria using 16S rRNA amplicon data.
4. Determine the location of the bacterial biofilms in the cooling section of the heat exchanger.

LITERATURE REVIEW

Continuously Operated Dairy Processing Equipment

Pasteurizing heat exchangers are typically operated on a continuous basis for more than 18h at a time. Continuously operated equipment also includes cream separators, membrane filtration systems, evaporators, and spray driers. Long operation periods without cleaning facilitates biofilm formation. Many of these are operated within a temperature range of 10 to 50°C (Bylund, 2003), which is within the commonly accepted food safety temperature "danger zone" of 4 to 60°C. Therefore, growth of bacteria during their operation should be expected to occur.

The presence of surviving bacteria from previous processing steps, along with time and temperature are critical factors that allow biofilms to form and increase in size and thickness. In processes such as pasteurization, some bacteria survive as it is not a sterilization process, that can then attach to the latter portions of the equipment and initiate biofilm formation. As the biofilm matures, this will eventually lead to the release of cells into the finished product. Abuse of these factors, such as temperature held within a range appropriate for bacterial growth for any period, allows bacterial growth and biofilm formation.

Fouling in Milk Heat Exchangers

Fouling consists of denaturation and adsorption of milk proteins and deposition of minerals, mainly calcium phosphate, particularly on the stainless-steel surfaces of dairy processing equipment. Minerals and proteins are the main components within fouling

material with fat only playing a minor part. Fouling is induced by a temperature differential where the proteins and minerals closest to the metal wall of the heat exchanger get hot enough to become insoluble. This creates a concentration gradient, which results in fouling as these components are deposited onto the wall (Walstra et al., 2005). Over time this reduces heat transfer from the heating media to the liquid milk causing problems with the milk reaching pasteurization temperature.

There are two types of fouling material that can form in milk heat exchangers, Type A and Type B. Type A forms at 80°C and is composed of 50-70% protein, 30-40% minerals, and 4-8% fat (Piepiórka-Stepuk et al., 2016). This type usually appears yellow and curd-like, and is associated with the denaturation of β-lactoglobulin. During milk pasteurization, temperatures in the fluid milk can reach up to 80°C, therefore, Type A fouling is likely to form. Type B fouling forms at temperatures above 110°C and is made up of 70% minerals, specifically calcium phosphate, and also includes some protein. Type B appears grey and grainy (Walstra et al., 2005).

Fouling does not tend to occur in the regeneration and the cooling sections because temperatures should not reach above 72°C. However, a monomolecular layer of proteins will form when milk encounters a metal surface. Biofilms form when bacteria attach to this monomolecular layer. Biofilms are incapable of forming in the heating section because of the higher temperature (Walstra et al., 2005).

Biofilms

Biofilms are composed of microbial communities embedded in a matrix of extracellular polymeric substances attached to a surface. Biofilm communities can be simple, composed of a few layers of bacteria surrounded by exopolysaccharide (**EPS**), or

they can be complex in structure, containing multiple bacterial layers with water channels that allow the movement of nutrients, metabolites, and waste products through the biofilm matrix (Sauer et al., 2007). Niches form within complex biofilms which allows various types of microorganisms, including aerobes and anaerobes, to thrive (creating microenvironments differing in oxygen levels, nutrient concentrations, and redox potential). The organisms in these biofilms are actively growing inside causing biofilms to increase in thickness over time, while microorganisms on the biofilm surface are in stationary phase, making biofilms resistant to cleaning treatments.

The basic steps of biofilm formation include the development of a conditioning film, attachment of bacterial cells, growth of cells with accompanying production of EPS, and, finally, biofilm maturation with release of cells into the environment. When a surface comes into contact with milk, a thin film composed of protein and fat forms within 10 s (Mittelman, 1998). This conditions the surface by causing changes in surface roughness and hydrophobicity which allow bacteria to attach more readily (Lorite et al., 2011). Free-floating bacterial cells in the fluid environment can attach to a conditioned surface initiating a biofilm, or to an existing biofilm, causing it to increase in bacterial number and thickness. When a biofilm matures, it begins to release cells into the surrounding aqueous environment. Bacterial cells can be released as individual cells, or in larger biofilm communities, entire sheets of biofilm can slough off into the surrounding environment (Horn et al., 2003).

Biofilms and the Dairy Industry

Environmental biofilms are ubiquitous in dairy processing plants, having been found in drains, on floors, belts, and seals (Costerton et al., 1995). Biofilms in the dairy

processing environment form due to the presence of constant water and nutrients and because dairy plants and pasteurized milk, which is a constant inoculum source, are not sterile. These environmental biofilms are generally not a direct threat to product contamination as they will not come into contact with the product itself.

Biofilms that form on food contact surfaces, however, are of the utmost importance as they provide opportunities for contamination of food products by potential pathogens or food spoilage organisms. Food contact surfaces in the dairy industry include production equipment, vats, milk tanks, piping, pasteurizing heat exchangers, evaporators, membrane filtration systems, and packaging machines. These areas often have conditions favorable for biofilm formation, such as the constant presence of moisture, nutrients, and microorganisms (Bower et al., 1996). Locations that permit biofilm development are areas that have a favorable environment for bacterial attachment and growth, as well as providing an adequate growth temperature. Such areas, which are often hard to clean adequately, include joints, cracks, corners, and valves (Storgards et al., 1999). Where these areas exist in a dairy processing plant, they must be cleaned regularly and thoroughly to prevent the buildup of any biofilm material.

Formation of Biofilms in Milk Pasteurizing Heat Exchangers

Pasteurization systems are composed of a heating section, holding tube, regeneration section, and a cooling section (Figure 1). The temperature within the heating section reaches up to 80°C, which can cause milk fouling where β-lactoglobulin proteins denature and form a monomolecular layer on the surfaces within the heat exchanger. This β-lactoglobulin layer continues to build up gradually over time with other proteins, molecules, and bacteria also adhering to the surface. Eventually, this layer leads to the

impedance of heat transfer, requiring higher temperatures to be used to achieve and maintain milk pasteurization temperature (De Jong, 1997).

The regeneration and cooling sections following the heating section have cools, it enters this temperature range, so cells and spores that survived pasteurization can attach to the conditioned surfaces within the regeneration and cooling sections and grow, temperatures that fall within the growth range for organisms found in milk. As the milk forming a biofilm. These sections of the pasteurizer will have the most biofilm growth, as this is where the optimum bacterial growth temperatures occur. Without rinsing or cleaning during long operating periods, biofilms can increase in thickness and release

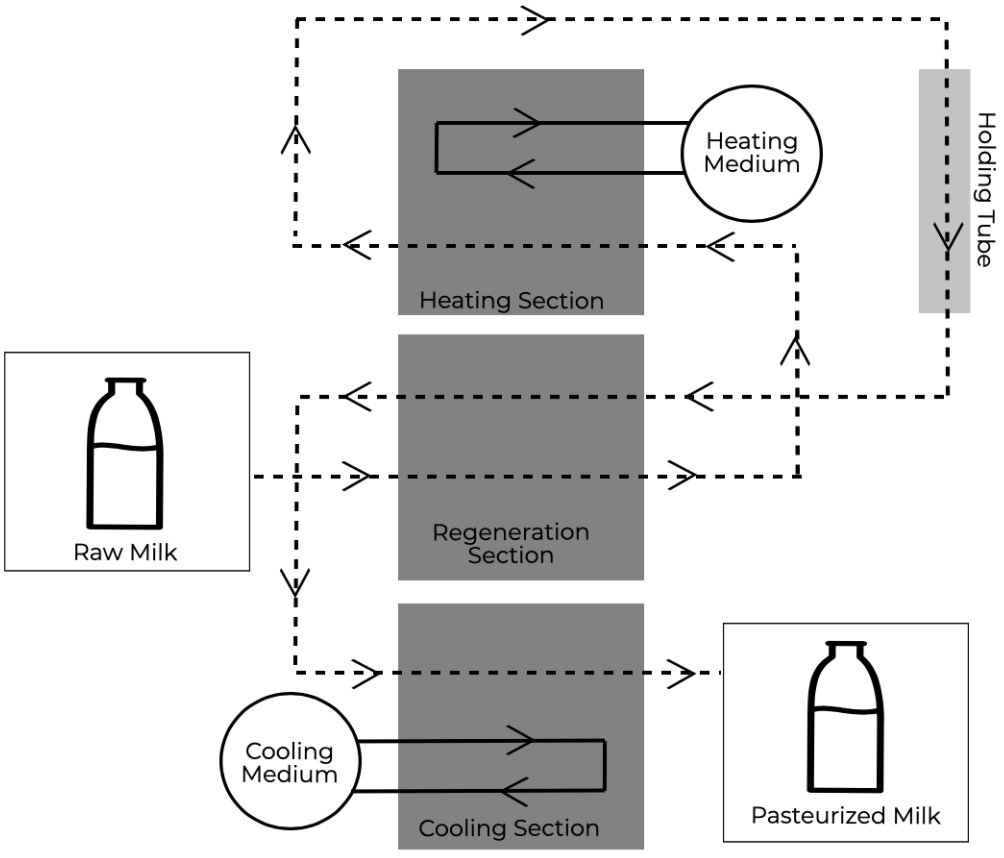

Figure 1. Diagram of industry-scale pasteurizer.

cells into the pasteurized product leading to increased bacterial load and reduced shelf life and quality (Burgess et al., 2009;Marchand et al., 2012). A 2018 study assessed the bacterial counts in milk as a function of time during 17-h pasteurization trials and found that there was an increase in bacterial counts in the pasteurized milk (Jindal et al., 2018). They also found biofilm material present on the heat exchanger plates within the regeneration section, which supports the theory that biofilm buildup can lead to increased bacterial counts in pasteurized milk (Jindal et al., 2018).

Raw Milk Microbiota

Milk is a nutrient-rich medium suitable for the growth of many different microorganisms. It is composed of water, lactose, fat, protein, and minerals which supply the nutrients required for a wide range of spoilage and even pathogenic bacteria. Bacteria typically found in raw milk include lactic acid bacteria (**LAB**), spoilage organisms (*Pseudomonas* spp. and *Bacillus* spp.), spore-formers, thermoduric bacteria, and pathogens (Ternström et al., 1993). Contamination of milk with microorganisms can occur either during or after milking. Sources of microbial contamination in milk include air, dust, feed, and bedding (Slaghuis et al., 1997;Te Giffel et al., 2002). Contamination can also originate from the cow itself as the microflora of the cow can be introduced into the milk during milking. The cow's udders and skin could harbor pathogens transferred to the milk during milking if the health of the cow is poor and if conditions on the farm are unsanitary. Unclean processing equipment and storage equipment can also introduce microbial contamination into raw milk (Te Giffel et al., 2002). It is imperative that all equipment that comes into contact with milk is cleaned effectively to remain free of pathogens.

Lactic acid bacteria usually dominate the bacterial flora in raw milk with LAB cocci more abundant in number than LAB bacilli in raw milk (Franciosi et al., 2009). Lactic acid bacteria are very important in the dairy industry as they are used to produce cheese, yogurt, and other fermented dairy foods. These are called starter cultures as they are intentionally added to milk when making these products. However, in addition to starter LAB added to cheese, nonstarter LAB (**NSLAB**) can be present in low numbers which increase in number during aging. The LAB present in raw milk are all considered NSLAB. These NSLAB can contribute flavor and texture attributes of the cheese, especially during aging. The presence of certain NSLAB can be beneficial to the overall acceptance of the cheese, while some NSLAB have been shown to produce gas which can cause problems with consumer acceptance as well as problems with cheese handling, such as shredding (Broadbent et al., 2003).

In a survey of bulk tank milk done by Jayarao et al. (2006), the authors found several pathogens in raw milk including Shiga toxin-producing *Escherichia coli*, *Yersinia enterocolitica*, *Salmonella enterica*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Campylobacter jejuni*. Thirteen per cent of all of the tested milk samples contained at least one of these pathogens. Fortunately, these pathogenic bacteria are not expected to be part of a biofilm in a pasteurizer as they are readily killed by pasteurization.

Many bacteria form endospores under stressful conditions as a means of survival. Spore-forming bacteria and their endospores are often present in raw milk and many can survive pasteurization. Possible sources of bacterial endospores include soil, bedding material, and silage (Driehuis, 2013). *Bacillus* species, particularly *Bacillus cereus* and *Bacillus sporothermodurans*, are the most common spore-forming bacteria found in raw

milk (Scheldeman et al., 2005). *Bacillus cereus* is an organism of concern for both spoilage and food safety as it can cause curdling in refrigerated pasteurized milk, and it can also produce both emetic toxins and enterotoxins causing food-borne illnesses (Beecher and Macmillan, 1991;Ehling-Schulz et al., 2004). Some *B. cereus* strains have been shown to form biofilms that protect the spores and vegetative cells, allowing them to survive harsh environments (Ryu and Beuchat, 2005;Wijman et al., 2007;Auger et al., 2009).

Clostridium species, another spore-forming genus, such as *C. sporogenes*, *C. butyricum*, C. *beijerinckii*, and *C. tyrobutyricum* have frequently been found in raw milk (Cremonesi et al., 2012). These *Clostridium* species are able to grow at refrigeration temperatures and have also been isolated from cheeses with late blowing defect, early gas defect, and flavor defects (Le Bourhis et al., 2005;Gómez-Torres et al., 2015).

Spoilage Bacteria

According to the United States Department of Agriculture and the Food and Drug Administration, organisms that have the highest threat of spoiling dairy products include thermoduric, thermophilic, psychrotrophic, and spore-forming bacteria. These are organisms of concern in the dairy industry because of the potential that thermoduric and spore-forming bacteria have to survive pasteurization and spray-drying temperatures. Therefore, these organisms are of concern in processing of milk powders and cheeses (Rückert et al., 2004;Scheldeman et al., 2006).

Bacillus, *Geobacillus*, and *Anoxybacillus* are the most common spore-forming genera found in milk powders. Scott et al. (2007) tested milk powders from 18 different countries, where 92% of the spores isolated were either *Bacillus licheniformis*, *G.*

stearothermophilus, or *Anoxybacillus flavithermus*. These organisms were also shown to form biofilms on stainless steel coupons in the presence of skim milk (Sadiq et al., 2017), indicating their potential to thrive in continuously operated equipment.

Streptococcus thermophilus is used as a starter culture in both Mozzarella and Cheddar cheeses for acid production in cheese manufacture. However, it is a thermoduric bacterium that can also survive pasteurization to colonize downstream equipment as a biofilm (Bouman et al., 1982;Flint et al., 1997). Knight et al. (2004) found *S. thermophilus* biofilms in the regeneration portion of a pasteurizing heat exchanger system. The presence of *S. thermophilus* in milk used for cheesemaking can cause off flavors and texture defects (Bouman et al., 1982).

The quality and shelf-life of products that use dairy powders as ingredients are detrimentally affected by the presence of thermophilic spores resulting in economic losses and food waste (Flint et al., 1997). Thermophilic spores, particularly *Bacillus* species, in powdered milk products could germinate if conditions allow after reconstitution, which can cause off-flavors caused by acid and enzyme production (both lipolytic and proteolytic enzymes). *Bacillus stearothermophilus* spores, which produce very heat stable proteolytic enzymes, are especially important as they were found to be more abundant in milk powders than in both raw and pasteurized milk (Chopra and Mathur, 1984).

Conclusion

Pasteurization is an important step in the processing of most dairy products to kill pathogens and reduce spoilage organisms. Biofilms can accumulate in the cooling section of pasteurizers through fouling and the presence of the organisms that survived

pasteurization. Continuously operating pasteurization equipment increases the likelihood of biofilm accumulation and eventual release of the cells into the pasteurized milk and, thus, the finished product. Bacteria in these biofilms could include NSLABs and spore-formers that could shed both vegetative cells and bacterial endospores. If these organisms are present in high numbers in a final product or milk used in further processing for cheese and milk powders, they can cause quality defects. Current research is deficient concerning the extent of biofilm formation and bacterial shedding during long-term pasteurization runs. There is very little information on what bacteria are harbored in and eventually released from biofilms formed in the regeneration and cooling portions in pasteurizing systems. Additionally, there have been very few studies concerning the bacterial count of milk as a function of time during long pasteurization runs. Keeping bacterial numbers low in fluid milk and milk products is vital to produce good quality milk. If the bacterial count of dairy products does increase significantly over time, quality would be compromised and money could be lost. It is to be determined whether longer processing times increase the bacterial load of pasteurized products.

MATERIALS AND METHODS

Heating Trials

Initial Setup. A lab-scale heat exchanger was assembled consisting of heating and cooling sections using 10 x 35 cm rubber-gasketed channel plates (4H T2B2-316-0.5-NBRP and 2H T2B2-316-0.5-NBRP) and end plates (II 2H T2B2-316-0.5-NBRP and I 2H T2B2-316-0.5-NBRP) from Statco Engineering (Huntington Beach, CA), custom built press plates, several peristaltic pumps (Masterflex, Cole-Parmer, Vernon Hills, Illinois), as well as hot and cold-water baths. Temperatures were recorded using T-type thermocouple probes (Omega, Norwalk, Connecticut) on inlet and outlet positions on the product and heating and cooling water. The temperature data was recorded using a data logger (34972A LXI Data Acquisition Unit, Keysight Technologies, Santa Rosa, California). All tubing used in initial trials was Masterflex® L/S® Precision Peroxide-Cured Silicone Pump Tubing Size 18 (Cole-Parmer).

Raw milk collected from the George B. Caine Dairy Teaching and Research Center (Utah State University, Wellsville, UT) and provided through the university's Aggie Creamery (Logan, UT), was allowed to warm to about ~15°C in stainless steel 38-L milk cans and then pumped into the system at 0.5 L/min using a peristaltic pump (Model 7522-28, Masterflex, Cole-Parmer). Milk entered the preheating section where it was heated to 65°C, then into the heating section where it was heated to 72°C. The heated milk then entered the holding tube where it was held at the heated temperature for 15 s. Milk entered the cooling section and then exited at 40°C. Water used as heating medium for the heating section was heated to 85°C in a 50 L hot water bath with a 1500W

Tempunit® thermoregulator (Model TU-20D, Techne, Cole-Parmer) and two additional 1500W heating elements (Model 290-3, Heetgrid Immersion Heater, George Ulanet Company). The heating medium was pumped into the preheating section using a peristaltic pump (Model 7520-40, Masterflex, Cole-Parmer). Cold water was used as the cooling medium and was pumped into the cooling section using a peristaltic pump (Model 7554-90, Masterflex, Cole-Parmer). The flow of product and heating or cooling media through the heat exchangers are shown in Figures 2 and 3. Equipment assembly is shown in Figure 4. Milk was only passed through the heat exchanger once (i.e., no recirculation) such that about 540 L of raw milk was processed during the 18-h pasteurizer run times.

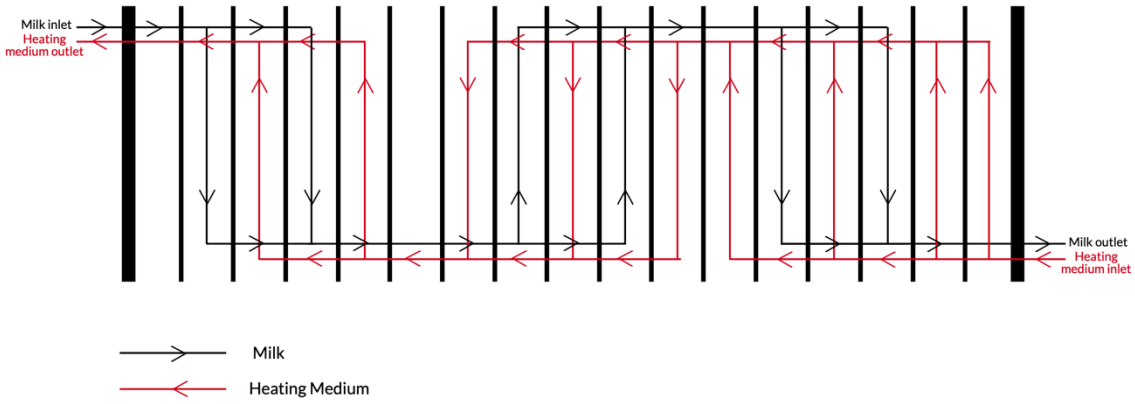

Figure 2. Configuration of the flow of milk and heating medium in the heating section heat exchanger.

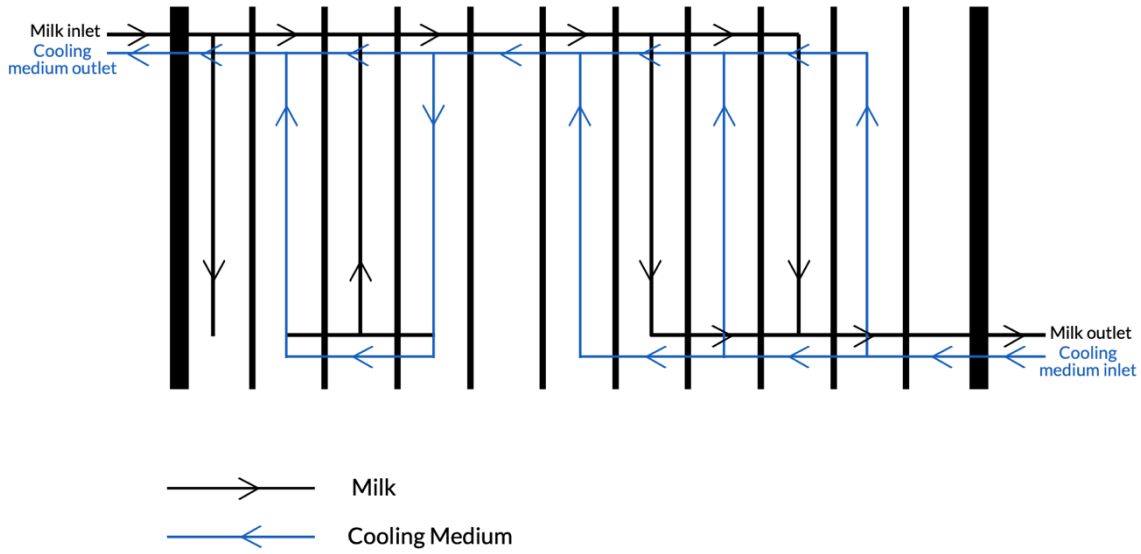

Figure 3. Configuration of the flow of milk and cooling medium in the cooling section heat exchanger.

Figure 4. Bench-top heat exchanger system setup. Left to right: heating medium water bath, heating medium pump, heating section, product pump, data logger and computer, cooling section, cooling medium pump, cooling medium reservoir.

Final Setup. A lab-scale heat exchanger was assembled and set up in the Gary Haight Richardson Dairy Products Laboratory at Utah State University (Logan, UT). It consisted of preheating, heating, and cooling sections using 10 x 35 cm rubber-gasketed channel plates (4H T2B2-316-0.5-NBRP and 2H T2B2-316-0.5-NBRP) and end plates (II 2H T2B2-316-0.5-NBRP and I 2H T2B2-316-0.5-NBRP) from Statco Engineering (Huntington Beach, CA), custom built press plates, several peristaltic pumps (Masterflex, Cole-Parmer, Vernon Hills, Illinois), as well as a cold-water bath. A 700 L horizontal cheese vat in the Dairy Products Laboratory was filled with water and heated to 75°C to provide the heating medium. Temperatures were recorded using T-type thermocouple probes (Omega, Norwalk, Connecticut) on inlet and outlet positions on the product and heating and cooling water. The temperature data was recorded using a data logger (34972A LXI Data Acquisition Unit, Keysight Technologies, Santa Rosa, California). Masterflex® L/S® Precision Peroxide-Cured Silicone Pump Tubing Size 18 (Cole-Parmer) was used for cooling medium and Masterflex® I/P® Precision Pump Tubing, Norprene (Cole-Parmer) was used for heating medium, holding tube, and milk inlet and outlet. The heat exchanger sections including inlet and outlet portions with thermocouple probes were insulated using denim insulation. The tubing for heating medium, raw milk, and the holding tube were all insulated using 2.5-cm foam pipe insulation.

Raw skim milk, collected from the George B. Caine Dairy Teaching and Research Center (Utah State University, Wellsville, UT) and provided through the university's Aggie Creamery (Logan, UT), was stored in a refrigerated holding tank and kept at 4°C. Prior to running raw milk through the pasteurizer, ~45-L batches of milk were pumped into steam-jacketed open cheese vats and heated to 40 to 50°C. The milk was pumped

into the system at 0.75 L/min using a peristaltic pump (Model 77411-00, Masterflex, Cole-Parmer). Milk entered the preheating section which maintained the batch-preheated temperature of 50°C then into the heating section where it was heated to 72°C. The heated milk entered the holding tube where it was held at the heated temperature for 15 s. Milk entered the cooling section and exited at 40°C. Temperature of the hot water was increased during processing to account for reduced heat transfer due to fouling in the heat exchanger. The heating medium was pumped into the preheating section using a peristaltic pump (Model 7520-40, Masterflex, Cole-Parmer). Cold water was used as the cooling medium and was pumped into the cooling section using a peristaltic pump (Model 7554-90, Masterflex, Cole-Parmer). The flow of product and hot water through the heating section was the same as shown in Figure 2 and through the cooling and pre-heating sections shown in Figures 3 and 5. A schematic of the pasteurizer system in the Dairy Products Laboratory is shown in Figure 6. About 810 L of raw milk was processed during the 18-h pasteurizer run times as the milk was only passed through the heat exchanger once (i.e., no recirculation).

Sample Collection and Preparation

During each initial and final trial, 50 mL samples of pasteurized-milk was collected from the outlet tubing every hour in a sterile 50-mL bottle. For final trials, 50 mL samples of preheated raw milk were taken every 6 hours. Samples were immediately cooled on ice, then transferred to refrigerated storage once their temperature reached 15°C. After 18 h of continuous operation, heating and cooling sections were dissembled. The entire surface area (200 cm^2) of each plate was swabbed using buffered peptone sponge sticks (3M, St. Paul, Minnesota).

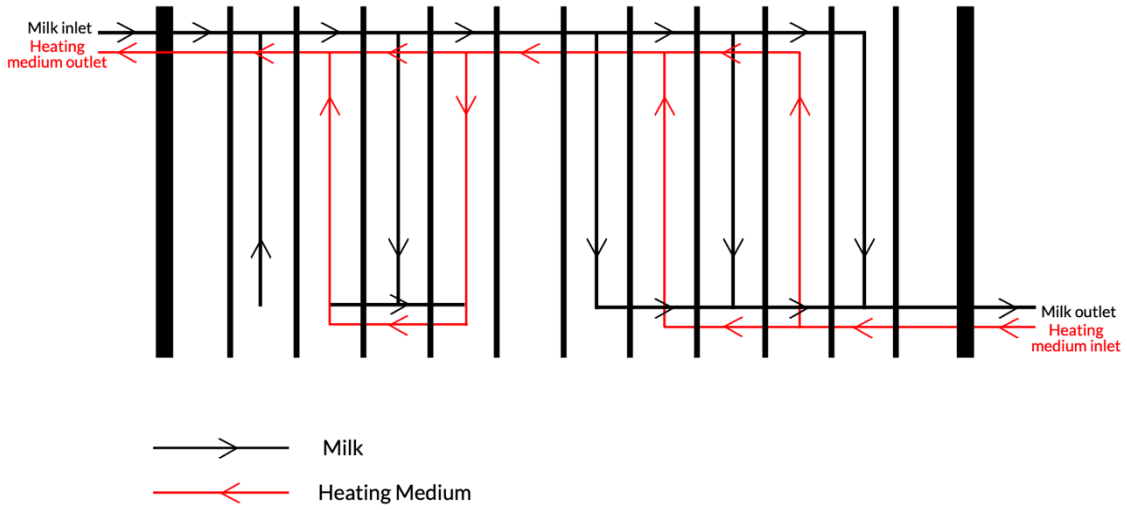

Figure 5. Configuration of the flow of milk and heating medium in the preheating section heat exchanger.

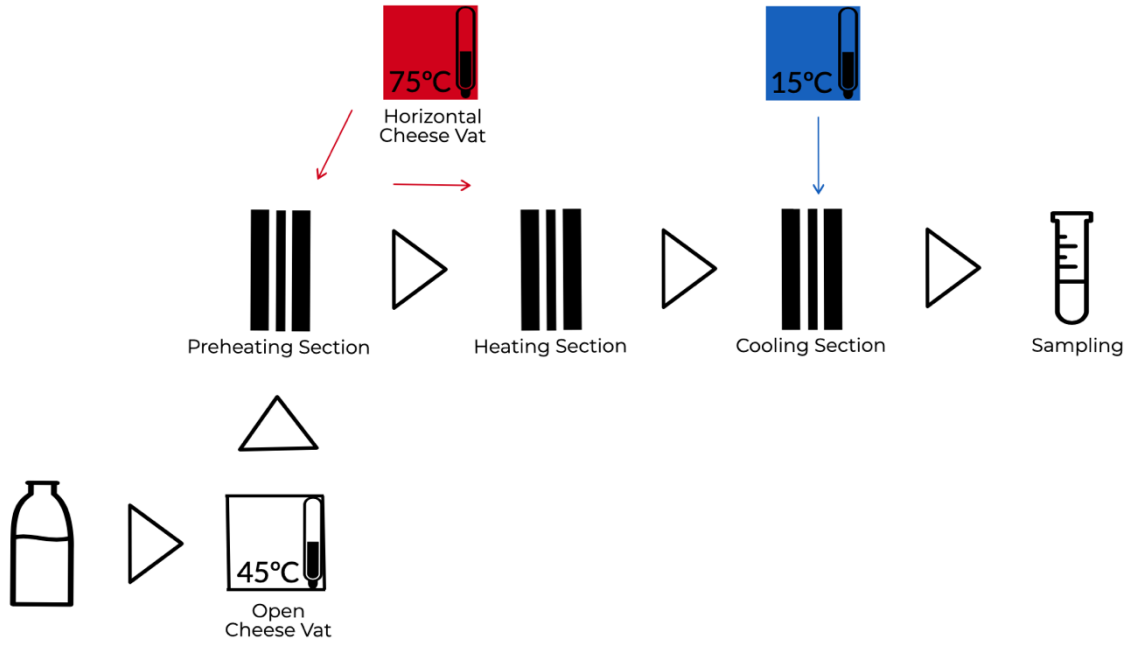

Figure 6. Schematic of pasteurizer setup in the Gary Haight Richardson Dairy Products Laboratory at Utah State University. Milk was heated in an open cheese vat, then pumped through the preheating and heating sections, the holding tube, and the cooling section. The heating medium was heated in a horizontal cheese vat and pumped through the preheating section then the heating section. The cooling medium was cold water from the Dairy Products Lab.

Enumeration of Bacteria

From our initial studies, total mesophilic counts were less than 100 CFU/mL for the first 11 h of continuous pasteurization. Therefore, no dilutions were performed on pasteurized milk collected up to 11 h. For raw milk and pasteurized milk after 12 h, serial dilutions to 10^{-4} were performed using sterile 9-mL distilled water dilution blanks. Then 0.1 mL aliquots of the milk and diluted samples were spread onto Standard Methods agar (**SMA**) (Hardy Diagnostics, Santa Maria, California) plates using sterile L-Shape Spreaders (VWR International, Radnor, PA) in triplicate. Inoculated plates were incubated for 48 h at 30°C. The same plating scheme was also used for total thermophilic enumeration with inoculated plates incubated at 55°C for 48 h.

Thermophilic spores were enumerated as detailed by Watterson et al. (2014) by first employing a spore pasteurization step to kill the vegetative cells. Samples were heated in sterile screw-capped glass tubes for 12 min at 80°C. All samples were serially diluted to 10^{-3}, then plated and incubated as for total counts. Inoculated sponge sticks were stored at 4°C for ≤72 h, stomached for 2 min at 260 rpm, then serially diluted to 10^{-3} and plated (in duplicate) as described for total mesophilic and thermophilic counts.

Identification of Bacteria

To determine what species of bacteria were found in the heat-treated milk and in the cooling section, colonies of bacteria with different morphologies were selected and isolated from the SMA plates. Each colony selected was isolated and grown in pure culture in Tryptic Soy broth (**TSB**) (Hardy Diagnostics). Stock cultures of each isolate were maintained at -80°C in TSB broth. To identify each isolate, DNA extraction was performed using DNA Purification Kit (Promega, Sunnyvale, CA). Polymerase chain

reaction procedures were performed according to (Broadbent et al., 2003) using primers UF_1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and UR_1 (5′-GCTGGCACGTAGTTAGCC-3′) and GoTaq DNA Polymerase (Promega). A MinElute PCR Purification Kit (Qiagen, Hilden, German) was used for PCR product purification. The purified PCR product was sequenced at the Genomics Laboratory at the Utah State University Center for Integrated Biotechnology. Sequences were then cleaned up by removing the primer sequences using 4Peaks version 1.7.2 (Mekentosj, Amsterdam, The Netherlands). The sequences were put into the National Center for Biotechnology Information (**NCBI**) Nucleotide Basic Local Alignment Search Tool (**BLAST**) for 16S ribosomal RNA sequences for Bacteria and Archae. The top 10 matches for each isolate sequence are included in Appendix A along with the sequence data.

Statistics

Repeated measures one-way ANOVA was used to assess statistical significance (α=0.05) using GraphPad Prism 8.3.1 for macOS (GraphPad Software, San Diego, California USA, www.graphpad.com). *P*-values for all statistics are included in Tables 1 and 2. *P*-values ≤ 0.05 were considered significant.

Table 1. P-values for raw and pasteurized milk samples

Raw and Pasteurized Milk Bacterial Counts	**p-value**
Raw Milk	0.1543
Pasteurized Milk - Mesophilic	0.1609
Pasteurized Milk - Thermophilic Total	0.0006*
Pasteurized Milk - Thermophilic Spore	0.6347

* indicates statistical significance ($P \leq 0.05$)

Table 2. *P*-values for heat exchanger samples

Heat Exchanger Bacterial Counts	**p-value**
Heating Section - Mesophilic	0.4165
Heating Section - Thermophilic	0.8277
Cooling Section - Mesophilic	0.6964
Cooling Section - Thermophilic	0.491

RESULTS AND DISCUSSION

Temperature

Initial Trials. The temperatures recorded over time at the end of the holding tube for each preliminary trial are shown in Figure 7. Even with warming the raw milk to 15 to 20°C and employing multiple heaters in the hot water bath, pasteurization temperatures were difficult to reach and maintain, as seen in Figure 7. In the beginning of each trial heating the milk to 72°C was achieved, but the temperatures dropped after ~1 hour and remained in the range of 66 to 70°C. This result indicates that the heat exchanger system could not supply the required heat to maintain pasteurization temperatures continuously for 18 h. This might be due to too much heat loss through the tubing and plate heat

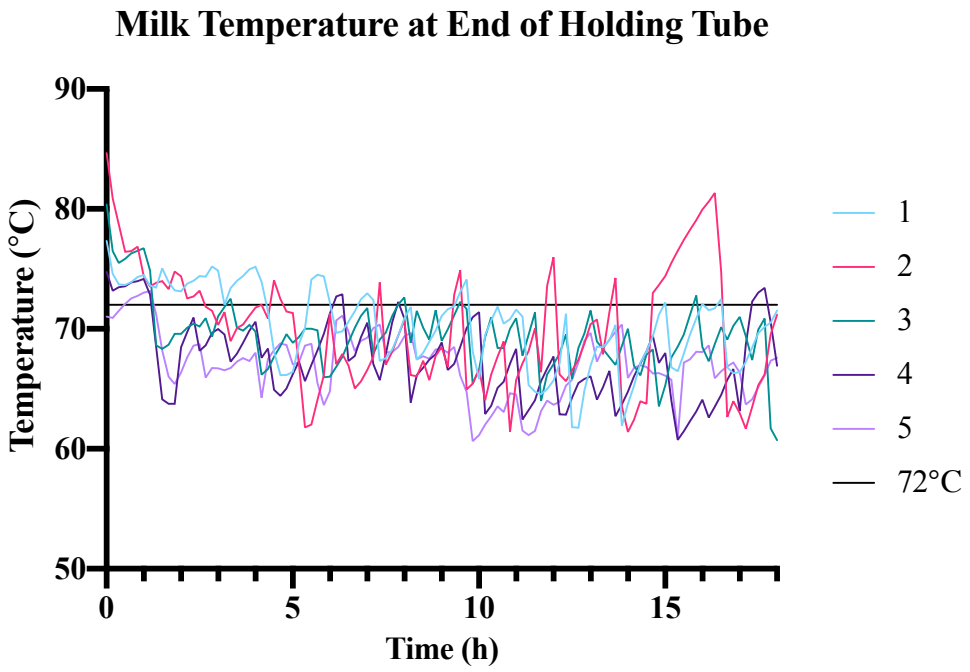

Figure 7. Recorded temperature (°C) at the end of the holding tube over time. A line for 72°C is shown for reference.

exchangers. The three heating elements provided 4,800 watts, and we needed approximately 4,500 watts to maintain the temperature of the water bath at 85°C in order to provide enough heat to reach and maintain pasteurization temperatures for the 18-h long trials. However, this amount of wattage was not enough to maintain the temperatures needed and the method of providing heat needed to be revised and better insulation added to reduce heat losses.

Final Trials. Pasteurization temperatures were difficult to achieve in both the initial pasteurizer configuration and the final configuration. The changes made to the initial setup were effective in maintaining pasteurization temperatures Heating the water (heating medium) in the horizontal cheese vat in the Aggie Creamery at USU, along with insulating most of the pasteurizing system, increased the amount of time during each trial that the milk was heated to 72°C or above. However, there were some instances during the final trials where the milk temperatures did drop below 72°C for <10 min at a time. This was due to air running through the system because of operator error. Overall, the temperatures were maintained at about 72°C. The temperature of milk at the end of the holding tube over time is shown in Figure 8.

Raw Milk

The raw milk mesophilic bacterial counts are shown in Figure 9. The bacterial counts increased substantially at the end of the trial, however, there was no significant difference ($p>0.05$). This could have been due to contamination of the raw milk samples, or the prolonged preheating of raw milk for longer than the previous preheated batches. The increase in bacterial count could have affected the bacterial numbers of the pasteurized milk. Raw milk samples were only taken every six hours, so it is unknown

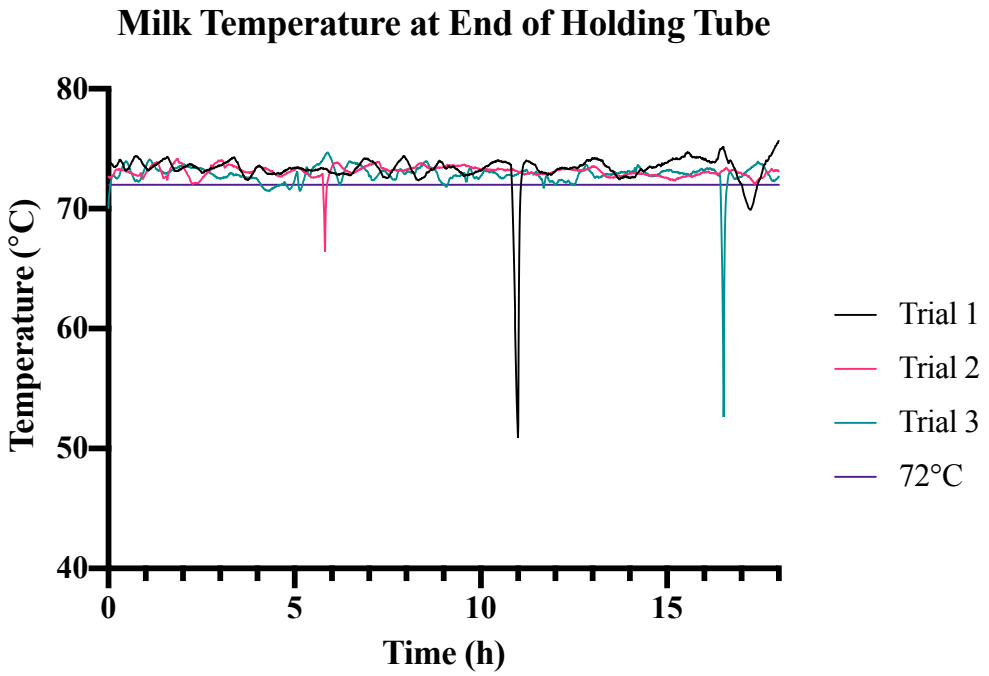

Figure 8. Recorded temperature (°C) of milk at the end of the holding tube over time during each trial. 72°C shown for reference.

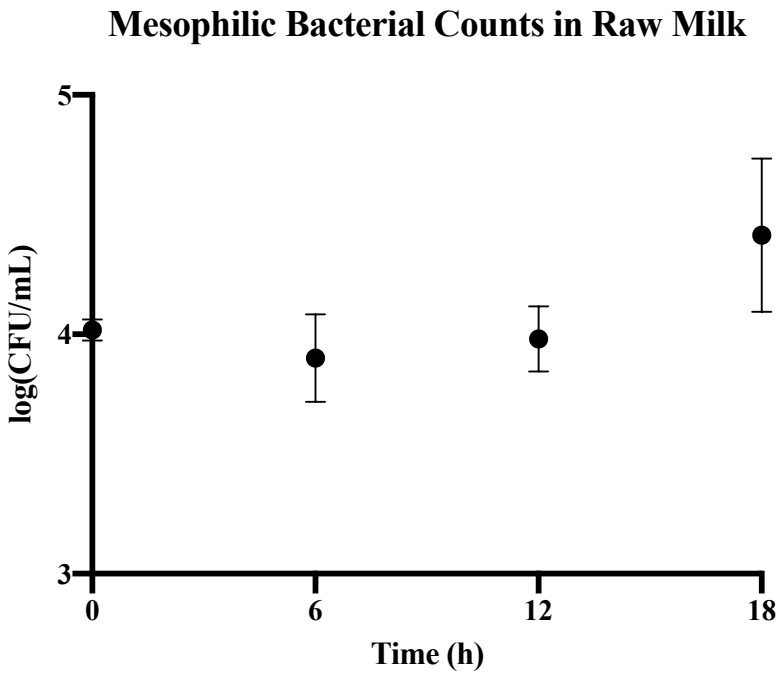

Figure 9. Mean ± SE for mesophilic bacterial counts in raw milk.

when the sharp increase in bacterial counts occurred. Typical raw milk bacterial counts in industry would be <100,000 CFU/mL as that is the Grade A raw milk standard.

Pasteurized Milk Counts

Mesophilic Bacterial Counts. In initial trials, there were low bacterial loads in the heat-treated milk (<5.0 x 102 colony-forming units (**CFU**)/mL) for most of the trials during the first ~10 h of processing (Figure 10). Bacteria counts were higher in four trials with an increase in numbers being observed after 9 h while in one trial there was no increase until after 15 h of processing. The bacterial counts in Trial 5 were about 1 log higher than the other four trials after 16 h. This is depicted as cumulative bacterial numbers based upon hourly measurements in Figure 10. Overall, the latter hours of each initial trial showed increased counts over time, with higher increases after 12 h.

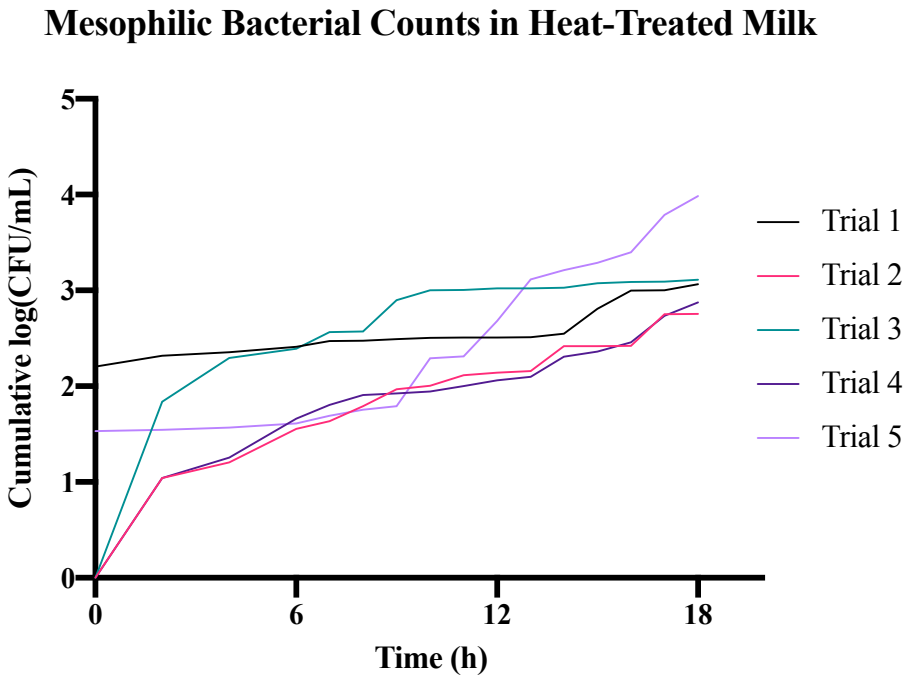

Figure 10. Cumulative bacterial load over time in initial trials.

Counts of mesophilic bacteria that survived pasteurization during the final trials are shown in Figure 11. During the first 16 h, bacterial numbers were very low. Bacterial counts increased 3-fold at 17 and 18 h. The presence of these mesophilic bacteria in the pasteurized milk samples suggests the samples were exposed consistently to contamination. The plate heat exchangers and tubing were not sterilized prior to each trial but rather sanitized with an acid sanitizer (AC 5-55 Red, Ecolab Inc., St. Paul, MN) as per industry procedure. The outlet tube was exposed to the environment and could have been a source of contamination. Another potential source of contamination could have been biofilm buildup in the milk outlet tubing. Even with the low levels of contamination, the mesophilic bacterial counts obtained from samples in the first 16 h were very low, $<6.0 \times 10^2$ CFU/mL. Grade A pasteurized milk bacteriological standards have a

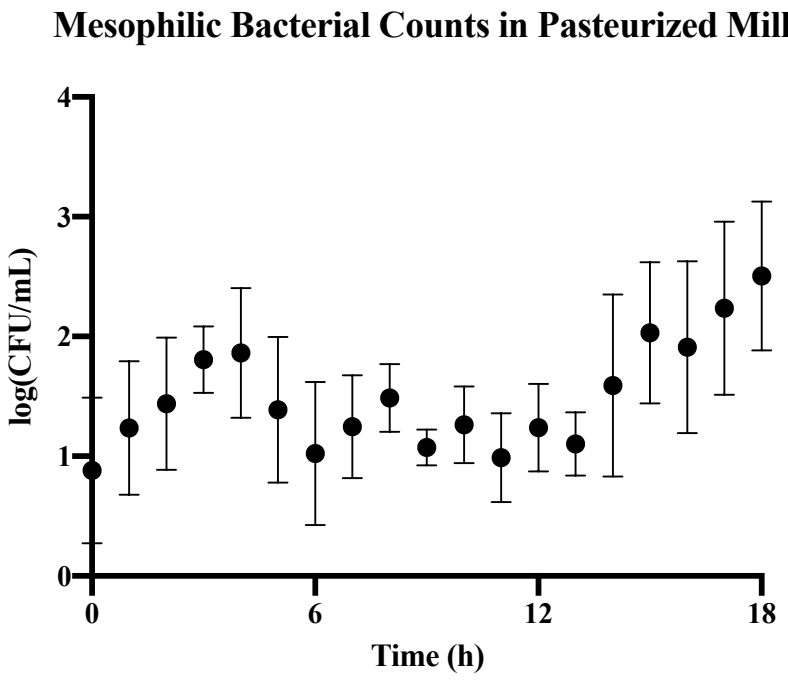

Figure 11. Mean ± SE bacterial counts over 18 h, n=3.

maximum limit of 20,000 CFU/mL (Grade "A" Pasteurized Milk Ordinance 2017). The substantial increase in mesophilic counts during the final 2 h suggests biofilm material built up enough in the plate heat exchangers or tubing to contaminate the pasteurized product. Interestingly, the trends for mesophilic bacterial count in initial and final trials was similar even with the sub-pasteurization temperatures (~68°C) in initial trials. The lower temperature treatment made little difference in bacterial counts. Jindal et al. (2018) found an increase in mesophilic bacterial counts first at 11 h and then again at 16 h with a sharper increase. This is similar to the trend of mesophilic bacterial counts in both the initial and final trials with increases starting after 11 h and then sharper increases in bacterial numbers at 16 h.

Thermophilic Bacterial Counts. Thermophilic bacteria that survived pasteurization increased after 15 h of pasteurization (Figure 12). This is similar to the trend observed for the mesophilic bacteria. For the first 7 h, the average thermophilic counts were less than 3.0×10^1 CFU/mL. There were subtle increases every other hour after 8 h. The average count for hour 8 was 4.1×10^2 CFU/mL, hour 9 was less than 3.0×10^1 CFU/mL, and hour 10 was up to 6.0×10^2 CFU/mL. This sporadic trend continued on through hour 16. After the 16^{th} hour, the counts increase consistently for two additional hours. The maximum thermophilic count was 8.2×10^3 CFU/mL at hour 18.

Thermophilic spore counts are shown in Figure 13. The first 16 h had averages less than 2.0×10^1 CFU/mL. At 16 h the spore counts went up and did not return to base levels. This is similar to both the mesophilic and thermophilic bacterial counts with respect to the trend and not the magnitude. The thermophilic spores were substantially lower than the thermophilic vegetative cell counts.

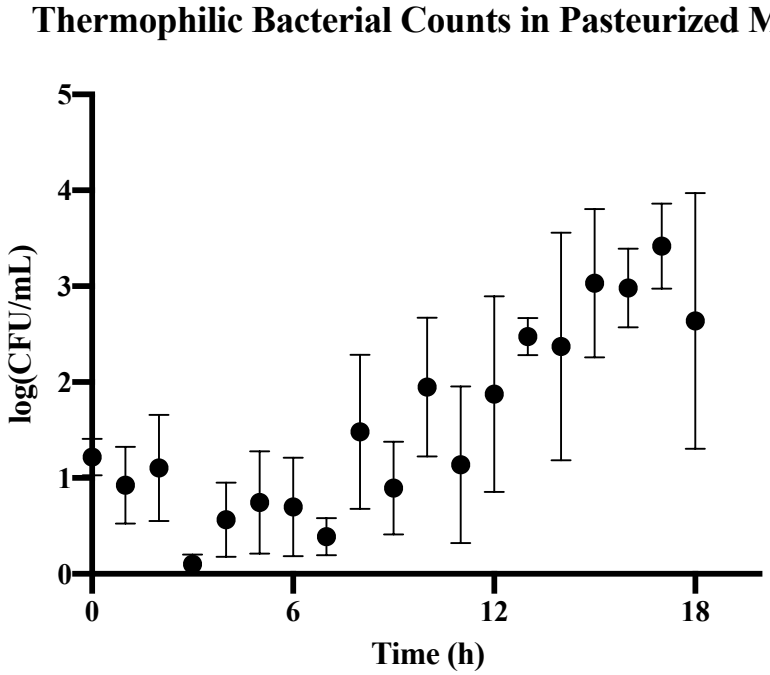

Figure 12. Mean ± SE for thermophilic bacterial counts over 18 h, n=3.

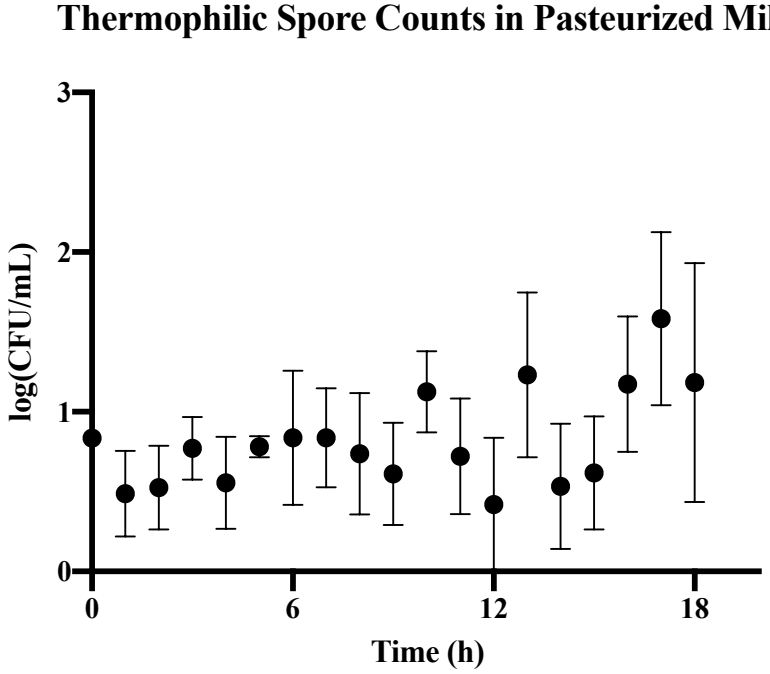

Figure 13. Mean ± SE for thermophilic bacterial spore counts over 18 h, n=3.

Fouling

In both the heating and preheating sections substantial fouling occurred and a layer of white/yellow curd-like fouling material was present (Figure 14). This was due to the high temperature differential between the heating medium and the raw product. The presence of the fouling material could have reduced the heat transfer from medium to product. Fouling material present in the plate heat exchangers could also harbor bacteria that can then contaminate downstream portions of the pasteurizer. There was no visible curd-like fouling material present in the cooling section which is due to the lower temperature within that plate heat exchanger section (Figure 14).

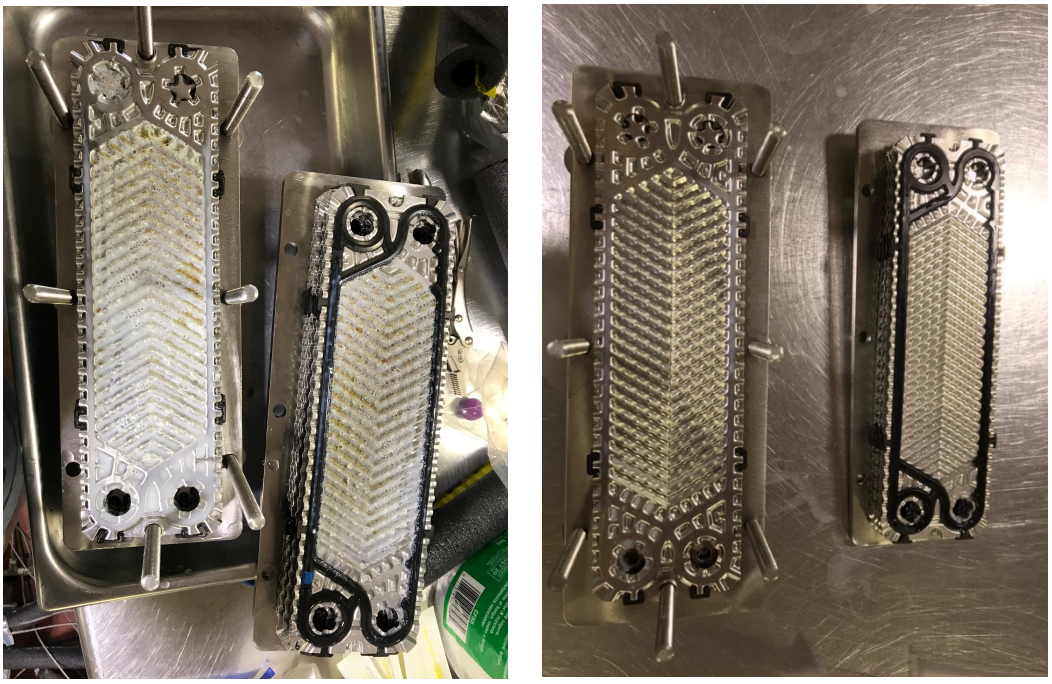

Figure 14. Left: heating section plate heat exchanger plates after 18-h pasteurization trial. Right: cooling section plate heat exchanger plates after 18-h pasteurization trial.

Plate Counts within Plate Heat Exchangers

Heating Section. Figure 15 shows mesophilic counts within the heating section. There were higher numbers of mesophilic bacteria toward the beginning of the heating section (plate U). The trend for thermophilic counts is the same as the mesophilic, higher at the beginning than at the end of the heating section (Figure 16). Milk temperatures in the heating section gradually increase from ~40°C to ~74°C, which explains the higher numbers of both mesophiles and thermophiles on the earlier plates in the plate heat exchanger as the temperature range for plates U and V is ~40-55°C (Figure 17). The temperature range for each plate in the heating section, and mesophilic and thermophilic counts are included in Table 3. At the end of the trials it was discovered that flow of milk through the preheating section was incorrect and milk was being held in the early portion at high temperatures for the entire duration of each trial. This incorrect flow could have led to an increase in the number of thermophilic bacteria harbored within the preheating section, thus increasing the bacterial count of the raw milk entering the heating section. Bacteria harbored in these early sections of the pasteurizer could contaminate downstream portions of the pasteurizer and accelerate biofilm buildup, which could lead to bacterial contamination of the pasteurized milk after about 11 h.

Cooling Section. The mesophilic bacteria on the cooling section plates in the final setup (Figure 18) also started off higher in the beginning of the heat exchanger with higher counts on plate A and lower on plate D. Surprisingly, the thermophilic plate counts within the cooling section followed the opposite trend (Figure 19). The counts were lower on plate A and highest on plates C and D. Flow of milk through the cooling section was also found to be incorrect, which could explain these trends (Figure 20).

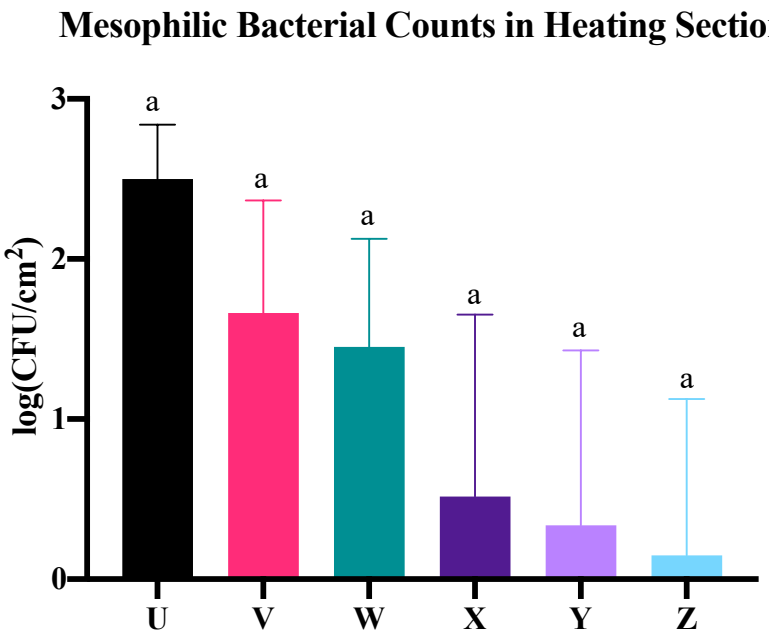

Figure 15. Mean ± SE for mesophilic bacterial counts on heating heat exchanger plates, n=3. The letters refer to plates within the heating section with plate U at the beginning of the heating section and plate Z at the end.

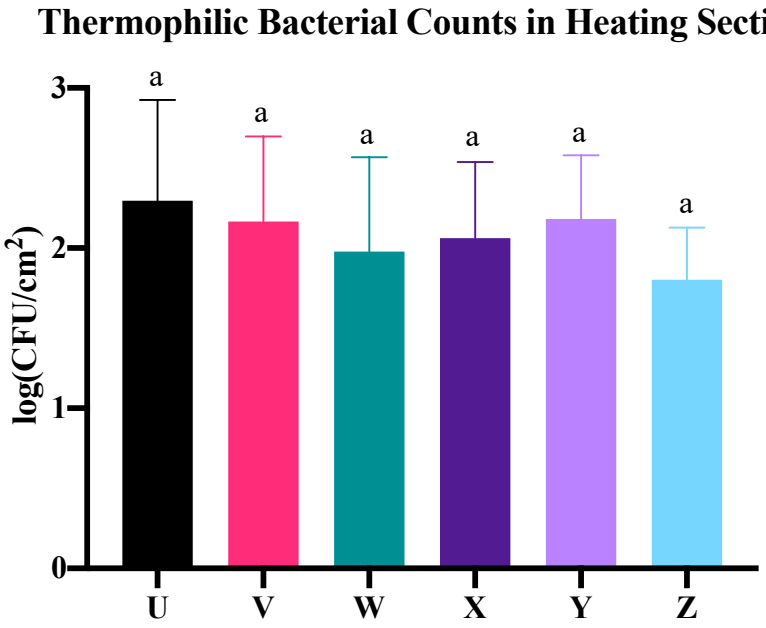

Figure 16. Mean ± SE for thermophilic bacterial counts on heating heat exchanger plates, n=3. Same letters as used in Figure 15. There was no statistical difference ($p>0.05$).

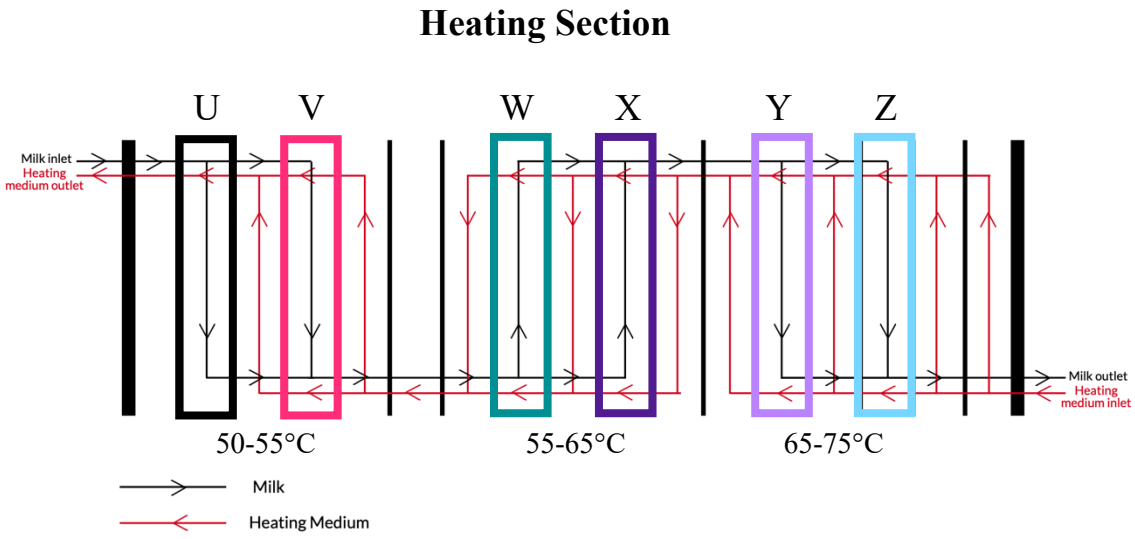

Figure 17. Temperature range of plates U – Z within heating section.

Table 3. Heating Section predicted temperature ranges, and bacterial counts on each plate

Plate	Predicted Temperature Range (°C)	Mesophiles Mean ± SE log CFU/mL	Thermophiles Mean ± SE log CFU/mL
U	40-50	2.5±0.3	2.3±0.6
V	50-55	1.7±0.7	2.2±0.5
X	55-65	1.5±0.7	2.0±0.6
W	55-65	0.5±1.1	2.1±0.5
Y	65-75	0.3±1.1	2.2±0.4
Z	65-75	0.1±1.0	1.8±0.3

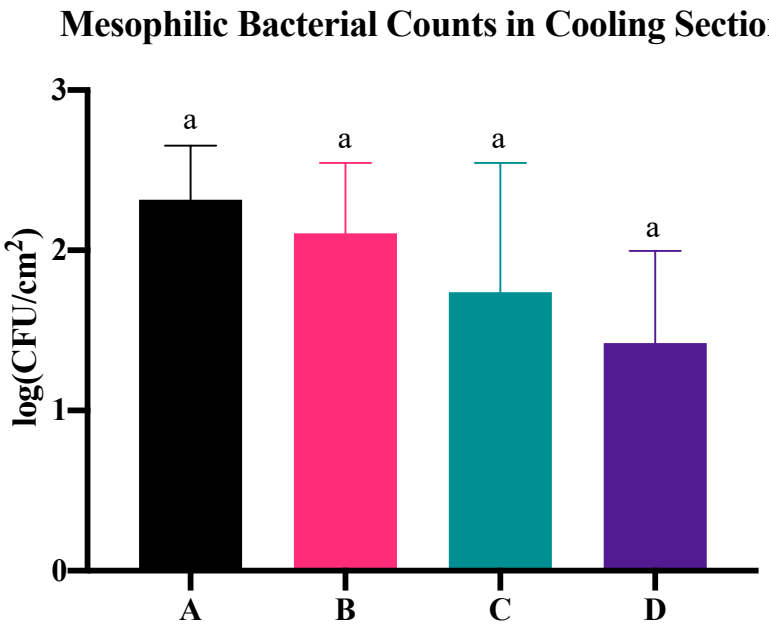

Figure 18. Mean ± SE for mesophilic bacterial counts on cooling heat exchanger plates, n=3. Letters refer to specific plates within the cooling section, plate A being at the beginning and plate D being at the end of the cooling section.

Flow through the first plates (A and B) was stagnant and the milk in contact with those plates was held at cooler temperatures (~40°C) for the entire duration of each trial. Mesophilic bacterial growth was higher than thermophilic bacterial growth on those first plates (A and B) due to the milk in contact being held at cooler temperatures. Jindal et al. (2018) enumerated the bacteria present on the heat exchanger plates following a 17-h pasteurization run and found that in the regeneration section there were ~log 1.5 CFU/cm^2 for both mesophiles and thermophiles. The regeneration section in that 2018 study is comparable to the cooling section in this study in terms of temperature range. They didn't specify where in the plate heat exchanger that they sampled. Table 4 contains the predicted temperature range, mesophilic and thermophilic log CFU/cm^2 within the cooling section. The mesophilic counts in the cooling section ranged from 2.3 (plate A) to

1.4 (plate D). The range for the thermophilic bacterial counts was 0.5 (plate A) to 1.8 (plate D). Our findings are similar to the values that Jindal et al. found in their 2018 study.

Bacterial Species

Single bacterial colonies from milk samples taken every hour during initial pasteurization trials that were isolated and identified using 16S rRNA sequencing are shown in Figure 21. Both *Bacillus* spp. and *Enterococcus* spp. are a part of the normal microbiota of raw milk (Scheldeman et al., 2006;McAuley et al., 2015). *Bacillus* species have the ability to form spores and survive pasteurization. *Enterococcus* spp. were present in all samples after 9 h. This might be because of environmental contamination and growth of bacteria in the outlet tubing. *Enterococcus* species are indicative of contamination in pasteurized milk products (Halkman and Halkman, 2014).

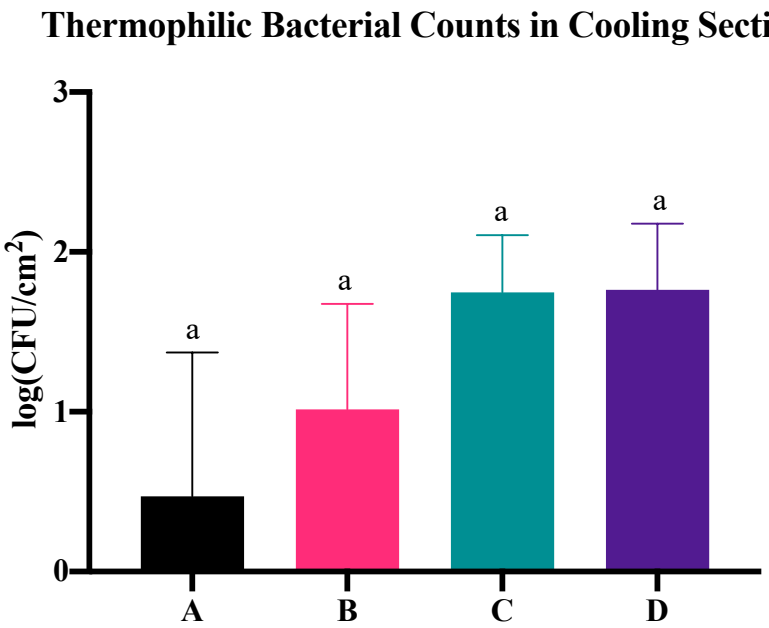

Figure 19. Mean ± SE for thermophilic bacterial counts on cooling heat exchanger plates, n=3. Same letters as used in Figure 19.

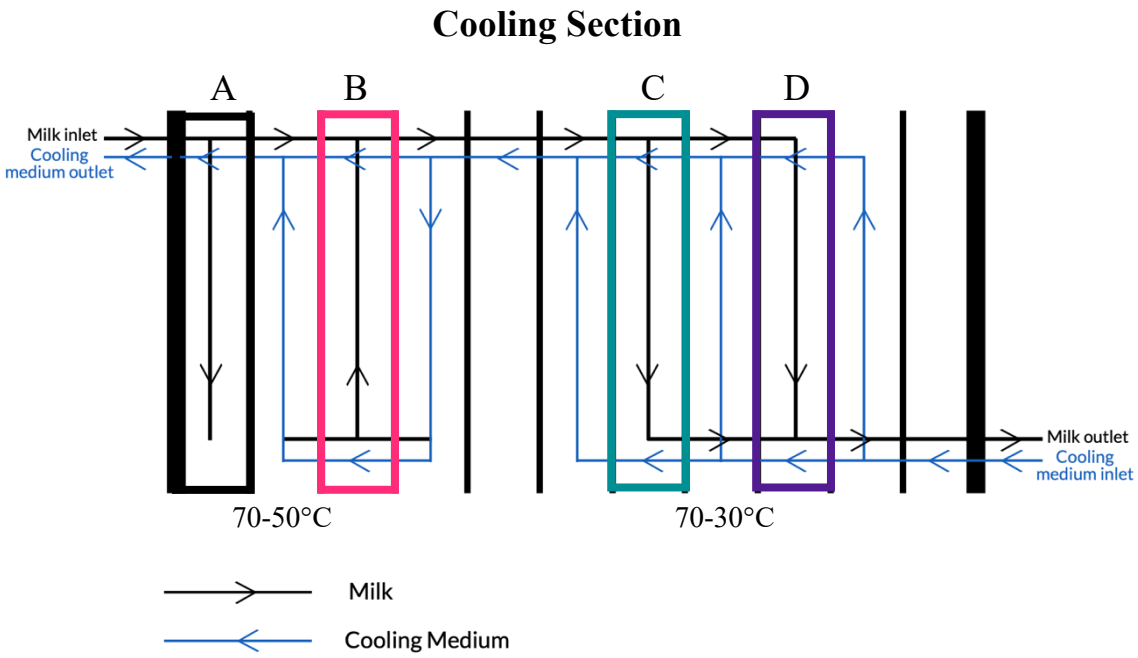

Figure 20. Temperature range of plates A – D within cooling section.

Table 4. Cooling section predicted temperature ranges, and bacterial counts on each plate

Plate	**Predicted Temperature Range (°C)**	**Mesophiles Mean ± SE log CFU/cm²**	**Thermophiles Mean ± SE log CFU/cm²**
A	70-40	2.3±0.3	0.5±0.9
B	70-40	2.1±0.4	1.0±0.7
C	70-30	1.7±0.8	1.7±0.4
D	70-30	1.4±0.6	1.8±0.4

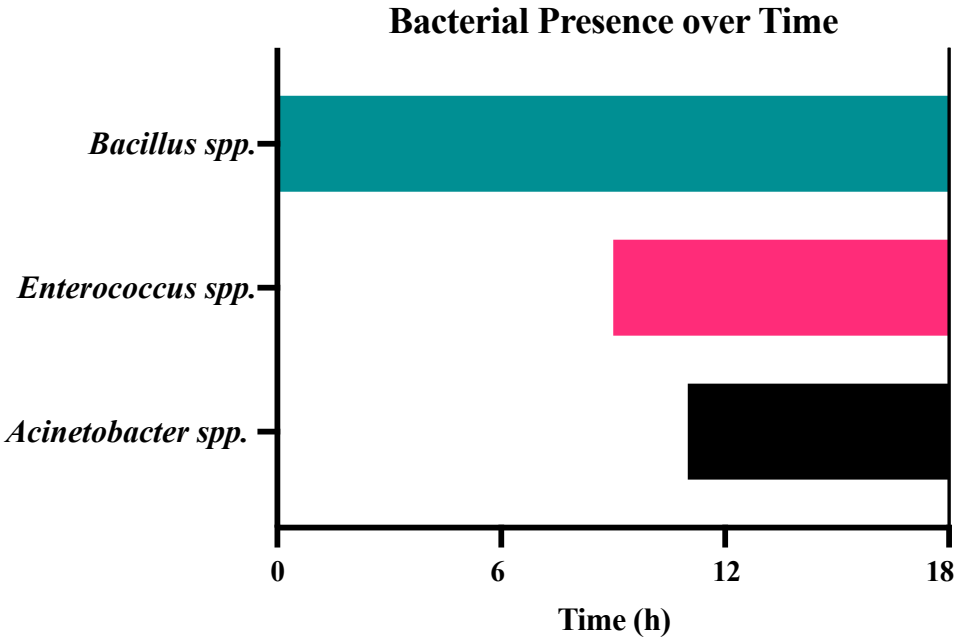

Figure 21. Mesophilic bacterial species found in pasteurized milk over time during one preliminary trial.

Acinetobacter spp. could be present in later samples likely due to contamination of outlet tubing. *Acinetobacter* species could be present in the plant environment as they are soil-borne bacteria considered ubiquitous (Cray et al., 2013).

The bacterial species that were isolated and identified are listed in Tables 5 and 6. Included in the table for each identified isolate are the ability to form spores and biofilms. Not surprisingly, all identified genera have also been found in raw milk. This indicates that the bacteria found in the pasteurized milk and on the plate heat exchangers could have been in the raw milk at low numbers. Additionally, the top 10 possible identities for each isolate, as well as the sequence data, are included in Appendix A.

The isolates found on the plate heat exchangers were also found in the pasteurized milk samples. There were only three bacterial species isolated from the heat exchanger plate samples, whereas, the pasteurized milk samples had 11 bacterial species identified.

There was little variation between the bacteria present on the heat exchanger plates. The pasteurized milk samples varied a lot in terms of bacterial isolates. This could have been due to contamination of the outlet tubing as there could have been biofilm buildup in causing contamination of the pasteurized milk.

Jindal et al. (2018) found *Bacillus* spp., *Pseudomonas* spp., and *Staphylococcus* spp. present in pasteurized milk samples and *Streptomyces* spp., *Staphylococcus* spp., *Bacillus* spp., *Brevibacillus* spp., *Kocuria* spp., and *Streptococcus* ssp. present in the biofilms on the plate heat exchanger plates in the regeneration section. In this study, *Bacillus* spp. and *Pseudomonas* spp. were also present in the pasteurized milk samples. *Bacillus* spp. and *Streptococcus* spp. were present in the cooling/regeneration section plate samples. The differences in these findings could stem from the difference in identification methods. Jindal et al. used Matrix-assisted laser desorption/ionization time of flight (**MALDI-TOF**) mass spectrophotometry, which examines ribosomal proteins, to identify the selected isolates from pasteurized milk and the surface of the plate heat exchangers (Jindal et al., 2018). In this study, 16S rRNA sequencing was used for bacterial identification. The methods could have produced different results. Additionally, the bacteria present in the raw milk prior to pasteurization could have been different, and the pasteurization systems and processing environments could also account for the differences in the species identified in each study.

Of bacterial isolates found in pasteurized milk and on plates within the cooling section, several are typically found in pasteurized milk in the United States (Ranieri and Boor, 2009). There were several isolates that are not typically found in pasteurized milk including, *Aneurinibacillus migulanus*, *Bacillus shackletonii*, *Brevundimonas*

vesicularus, *Moraxella osloensis* (Tables 5 and 6). These bacteria could have come from the processing environment and contaminated the raw milk or the pasteurized product.

Acinetobacter spp. have been found in dairy cattle, raw milk, and even in dairy processing facilities (Poirel et al., 2012;Gurung et al., 2013;Wang et al., 2019). It has been found to cause ropiness of milk and also secrete enzymes at psychrophilic and mesophilic temperatures. *Acinetobacter* spp. are found in high levels in raw milk and produce a capsular polysaccharide, which is the cause of the ropiness of milk and also the cause of slimy surface defects in cheese (Gennari et al., 1992). Though not typically biofilm-formers, certain species are able to form biofilms. *Acinetobacter baumannii* and *Acinetobacter junii* were constituents of biofilms formed on dairy surfaces (Wang et al., 2019). In a different study the following *Acinetobacter* species were found in biofilms in milking machines, *Acinetobacter albensis*, *Acinteobacter guillouiae*, *Acinetobacter johnsonii*, and *Acinetobacter parvus* (Weber et al., 2019). *Acinetobacter* spp. were found within biofilms associated with spiral-wound milk processing membranes (Chamberland et al., 2017).

Certain species of the genus *Aneurinibacillus* contain genes responsible for exopolysaccharide biosynthesis and biofilm formation (Alenezi et al., 2017). *Aneurinibacillus* spp. form highly heat resistant spores found in both silage and raw milk (te Giffel, 2002).

The *Bacillus* genus contains species that are psychrotropic, mesophilic, and thermophilic. *Bacillus shakletonii,* a thermophilic spore-former isolated from traditional Naan sourdough in northern China (Turpan, Xinjiang, China) and also from volcanic soil

Table 5. Bacterial species present in pasteurized milk samples

Genus	Mesophilic / Thermophilic	Spore-former	Ability to form biofilms
*Acinetobacter beijerinckii**	M	No	Not typically
Aneurinibacillus migulanus	T	Yes	Yes
Bacillus shakletonii	T	Yes	Yes
*Bacillus thuringiensis**	M	Yes	Yes
*Bacillus subtilis**	M	Yes	Yes
Brevundimonas vesicularis	M	No	Yes
Chryseobacterium scophthalmum	M	No	Yes
*Microbacterium aurum**	M	No	Yes
Moraxella osloensis	M	No	Yes
*Pseudomonas stutzeri**	M	No	Yes
*Streptococcus equinus**	M	No	Yes

*Indicates presence in pasteurized milk (Ranieri and Boor, 2009)

Table 6. Bacterial species present on heat exchangers plates within the cooling section

Genus	Mesophilic / Thermophilic	Spore-former	Ability to form biofilms
*Bacillus thuringiensis**	M	Yes	Yes
Moraxella osloensis	M	No	Yes
*Pseudomonas stutzeri**	M	No	Yes

*Indicates presence in pasteurized milk (Ranieri and Boor, 2009)

on Candlemas Island, South Sandwich archipelago (Logan et al., 2004;Kè and Fǔ, 2017), has been shown to be able to hydrolyze casein (Logan et al., 2004). *Bacillus subtilis*, a mesophilic microorganism, is able to form both spores and biofilms (Hilbert and Piggot, 2004;Mielich-Süss and Lopez, 2015). The biofilms created by *Bacillus subtilis* are well structured with fruiting bodies that enhance sporulation. Within the pasteurizer, it means that the biofilms of this bacteria could potentially be releasing spores into the end product due to the fruiting bodies (Branda et al., 2001). The following *Bacillus* species were also found in these biofilms within milking machines, *Bacillus clausii*, *Bacillus idriensis*, *Bacillus marisflavi*, *Bacillus paralicheniformis*, *Bacillus safensis*, *Bacillus simples*, and *Bacillus thuringiensis* (Weber et al., 2019). *Bacillus thuringiensis* is an efficient biofilm former (Verplaetse et al., 2017).

Brevundimonas spp. are not spore-formers and are found in water in dairy farms (Hervert et al., 2016). *Brevundimonas* spp. produce EPS so they are able to form biofilms (Verhoef et al., 2002). *Brevundimonas vesicularis* was isolated from biofilms within milking machines (Weber et al., 2019). In addition, it's found in water used in dental equipment and in water from paper factories (Verhoef et al., 2002;Szymanska, 2007).

Chryseobacterium spp. are found in dairy processing environments and also from raw milk itself. *Chryseobacterium joostei* and *Chryseobacterium indologenes* in particular were isolated from raw milk tankers and milking machines (Hugo et al., 2003;Weber et al., 2019). *Chryseobacterium haifense* is a psychrotolerant bacterium isolated from raw milk. The *Chryseobacterium* genus has importance in the dairy industry as species within this genus have been known to produce proteases that cause defects in dairy foods (Hugo et al., 1999;Hugo et al., 2003).

Some of the most common thermoduric bacteria found in dairy products are the species in the non-spore-forming genus *Microbacterium*. Several *Microbacterium* spp. including *Microbacterium lacticum*, *Microbacterium foliorum*, *Microbacterium luteolum*, *Microbacterium maritypicum*, and *Microbacterium testaceum*, were isolated from biofilms within dairy milking machines (Weber et al., 2019).

Moraxella spp. have been found in bulk-tank milk and in an ice cream processing facility (Jayarao and Wang, 1999;Gunduz and Tuncel, 2006). *Moraxella osloensis* was also found to be a part of the biofilm communities within milking machines (Weber et al., 2019).

Pseudomonas spp. (*Pseudomonas azotoformans, Pseudomonas congelans, Pseudomonas extremorientalis, Pseudomonas gessardii, Pseudomonas koreensis, Pseudomonas paralactis,* and *Pseudomonas poae*) were part of biofilm communities within milking machines and also in bulk-tank milk (Jayarao et al., 2006;Weber et al., 2019). In an ice cream factory the biofilm on belt of packaging machine harbored *Pseudomonas* spp. (Gunduz and Tuncel, 2006).

Streptococcus spp. were found within biofilms associated with spiral-wound milk processing membranes (Chamberland et al., 2017). *Streptococcus thermophilus* is used as a starter culture in mozzarella cheese. In the presence of milk proteins, *S. thermophilus* has been shown to be able to form biofilms (Bassi et al., 2017).

There were only three bacterial species identified on the plate heat exchangers compared to the 11 species identified in the pasteurized milk. This could indicate that there was contamination of the pasteurized milk after the cooling section because of the additional bacterial species found. The contamination of the pasteurized milk may have

been due to contamination of the sampling outlet tube. To remedy this problem, it would be beneficial to cut off the 6 inches of the outlet tube every three hours to avoid any buildup of contaminants.

CONCLUSION

This study investigated the effect of running a lab-scale pasteurizer for extended periods (18 h) on bacterial counts in the pasteurized milk and on the heat exchanger plates. Bacterial counts did increase by the latter end of the trials (16 h). The raw milk counts at the 18 h final sampling were higher than the earlier samples, however, there was no significant difference ($p>0.05$). There was a sporadic fluctuation in the bacterial counts throughout the pasteurization, which could be due to the small-scale system or due to the sporadic nature of biofilm coming off and entering the pasteurized milk. Spikes and fluctuation of bacterial counts are even typical in industry-scale pasteurized milk.

The bacterial counts on the heat exchanger plates followed an interesting trend, with higher bacterial counts toward the beginning of each heat exchanger (consistent for both heating and cooling sections). An exception to this trend being the thermophilic counts in the cooling heat exchanger that were lower toward the beginning of the section and higher at the end. This can be explained by the incorrect flow of the cooling section which only allowed the flow of milk through the latter two plates (plates C and D) in contact with the milk. The first two plates in contact with the milk (plates A and B) were setup such that the milk could only fill those portions up without flow through to the latter portions. The milk in these first portions was stagnant for the entire duration of each trial. This would explain the low thermophilic and high mesophilic counts in that area.

Bacterial species isolated from the plates were also isolated from the pasteurized milk, which could indicate sloughing of any biofilm material present. There were several

other bacterial species identified in the pasteurized milk which indicate there were contamination issues in the sampling method. The bacteria found on the plate heat exchanger plates and in the pasteurized milk have also been found in both raw and pasteurized milk according to literature, so it is not surprising to see these bacterial species in the samples.

My suggestions for future work would be to have complete flow through the preheating and cooling sections, to implement a contamination-control protocol where the end of the sampling tube is trimmed by 6 inches every 3 hours. I would also suggest removing the batch preheating of the raw milk as it would not have been necessary if the preheating section were setup so that there was complete flow through the section. Another change to implement would be to spike the raw milk using a spore culture to overcome the very low raw milk bacterial numbers. I would suggest comparing the lab-scale results with an industry-scale pasteurization run to better understand if the lab-scale system affects the trend in anyway.

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APPENDICES

APPENDIX A. BACTERIAL ISOLATE 16S RRNA SEQUENCE DATA

Isolate code: AT
UF1 sequence:
GAGGTTAGCGGCGGACGGGTGAGTAACACGTAGGCAACCTGCCTGTACGACC
GGGATAACTCCGGGAAACCGGAGCTAATACCGGATAGGATGCCGAACCGCA
TGGTTCGGCATGGAAAGGCCTTTGAGCCGCGTACAGATGGGCCTGCGGCGCA
TTAGCTAGTTGGTGGGGTAACGGCCTACCAAGGCGACGATGCGTAGCCGACC
TGAGAGGGTGAACGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACG
GGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAA
CGCCGCGTGAGTGAGGAAGGTCTTCGGATCGTAAAACTCTGTTGTCAGGGAA
GAACCGCCGGGATGACCTCCCGGTCTGACGGTACCTGACGAGAAAGCCCCGG
C

UR1 sequence:
CCCGGCGGTTCTTCCCTGACAACAGAGTTTTACGATCCGAAGACCTTCCTCAC
TCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTG
CTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGTTCACC
CTCTCAGGTCGGCTACGCATCGTCGCCTTGGTAGGCCGTTACCCCACCAACTA
GCTAATGCGCCGCAGGCCCATCTGTACGCGGCTCAAAGGCCTTTCCATGCCG
AACCATGCGGTTCGGCATCCTATCCGGTATTAGCTCCGGTTTCCCGGAGTTAT
CCCGGTCGTACAGGCAGGTTGCCTACGTGTTACTCACCCGTCCGCCGCTAACC
TCAGGAATGCAAGCACTCCATCGGTTCGCTCGACTTGCATGTATTAGGCACGC
CGCCAGCGTTCGTCCTGAGC

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Aneurinibacillus migulanus strain DSM 2895 super7, whole genome shotgun sequence	606	606	99%	2.00E-170	93.22%	NZ_LGUG01000004.1
Aneurinibacillus tyrosinisolvens strain LL-002, whole genome shotgun sequence	580	580	100%	1.00E-162	92.05%	NZ_BBWZ01000108.1
Rubeoparvulum massiliense strain mt6, whole genome shotgun sequence	520	520	98%	3.00E-144	89.71%	NZ_CVPE01000004.1
Bacillus indicus strain DSM 16189 Contig19, whole genome shotgun sequence	512	512	98%	4.00E-142	89.23%	NZ_JNVC02000019.1
Bacillus pumilus strain SH-B9, complete genome	490	3918	100%	2.00E-135	88.03%	NZ_CP011007.1

Aeribacillus pallidus strain 8m3 NODE_1, whole genome shotgun sequence	488	488	87%	7.00E-135	90.91%	NZ_LWBR01000013.1
Bacillus gobiensis strain FJAT-4402 chromosome	486	3877	99%	3.00E-134	87.79%	NZ_CP012600.1
Bacillus amyloliquefaciens DSM 7 = ATCC 23350, complete sequence	484	4760	88%	9.00E-134	90.54%	NC_014551.1
Bacillus glycinifermentans isolate BGLY genome assembly, chromosome: 1	483	3837	87%	3.00E-133	90.54%	NZ_LT603683.1
Bacillus licheniformis DSM 13 = ATCC 14580, complete sequence	483	3350	87%	3.00E-133	90.54%	NC_006270.3

Isolate code: BT
UF1 sequence:
CGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACGCC
GGGAAACCGGGGCTAATACCGGATAGTTTTTTCCTCCGCATGGAGGAAAAAG
GAAAGGCGGCTTCGGCTGCCACTTACAGATGGGCCCGCGGCGCATTAGCTAG
TTGGCGGGGTAACGGCCCACCAAGGCAACGATGCGTAGCCGACCTGAGAGG
GTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTG
AGTGAAGAAGGCCTTCGGGTCGTAAAACTCTGTTGCCGGGGAAGAACAAGTG
CCGTTCGAACAGGGCGGCGCCTTGACGGTACCCGGCCAGAAAGCCACGGCT

UR1 sequence:
GGCACTTGTTCTTCCCCGGCAACAGAGTTTTACGACCCGAAGGCCTTCTTCAC
TCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTG
CTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACC
CTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGGGCCGTTACCCCGCCAACTA
GCTAATGCGCCGCGGGCCCATCTGTAAGTGGCAGCCGAAGCCGCCTTTCCTTT
TTCCTCCATGCGGAGGAAAAAACTATCTGGTATTAGCCCCGGTTTCCCGGCGT
TATCCCAGTCTTACAGGCAGGTTGCCCACGTGTTACTCACCCGTCCGCCGCTA
ACCTTTTAAAAGCAAGCTTTTAAAAGGTCCGCACGACTTGCATGTATTAGGCA
CGCCGCCAGCGTTCGTCCTG

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacillus acidicola strain FJAT-2406 Scaffold1, whole genome shotgun sequence	588	5785	100%	7.00E-165	92.27%	NZ_KV440953.1
Bacillus methanolicus MGA3, complete genome	582	5228	100%	3.00E-	92.09%	NZ_CP007739.1

				163		
Quasibacillus thermotolerans strain SGZ-8 Contig10, whole genome shotgun sequence	577	577	100%	1.00E-161	91.83%	NZ_JWJE02000010.1
Bacillus shackletonii strain LMG 18435 scaffold8, whole genome shotgun sequence	571	571	100%	7.00E-160	91.59%	NZ_LJJC01000011.1
Bacillus shackletonii strain LMG 18435 super19, whole genome shotgun sequence	571	2286	100%	7.00E-160	91.59%	NZ_LJJC01000006.1
Bacillus marisflavi strain JCM 11544 Contig2, whole genome shotgun sequence	571	571	100%	7.00E-160	91.55%	NZ_LGUE01000011.1
Bacillus smithii strain DSM 4216, complete genome	571	6289	100%	7.00E-160	91.55%	NZ_CP012024.1
Bacillus campisalis strain SA2-6 scf7180000001092, whole genome shotgun sequence	569	569	100%	2.00E-159	91.55%	NZ_LAYY01000014.1
Bacillus vietnamensis NBRC 101237, whole genome shotgun sequence	566	566	100%	3.00E-158	91.37%	NZ_BCVQ01000102.1
Bacillus atrophaeus strain SRCM101359 chromosome, complete genome	566	4474	100%	3.00E-158	91.30%	NZ_CP021500.1

Isolate code: DT
UF1 sequence:
CTTGCTTTTAAAAGGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTG
CCTGTAAGACCGGGATAACGCCGGGAAACCGGGGCTAATACCGGATAGTTTT
TTCCTCCGCATGGAGGAAAAAGGAAAGGCGGCTTCGGCTGCCACTTACAGAT
GGGCCCGCGGCGCATTAGCTAGTTGGCGGGGTAACGGCCCACCAAGGCAAC
GATGCGTAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACACGG
CCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAA
GTCTGACGGAGCAACGCCGCGTGAGTGAAGAAGGCCTTCGGGTCGTAAAACT
CTGTTGCCGGGGAAGAACAAGTGCCGTTCGAACAGGGCGGCGCCTTGACGGT
ACCCGGCCAGAAAGCCACGGCTA

UR1 sequence:
CGACGGCACTTGTTCTTCCCCGGCAACAGAGTTTTACGACCCGAAGGCCTTCT
TCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCT
ACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGAT

CACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGGGCCGTTACCCCGCCA
ACTAGCTAATGCGCCGCGGGCCCATCTGTAAGTGGCAGCCGAAGCCGCCTTT
CCTTTTTCCTCCATGCGGAGGAAAAAACTATCTGGTATTAGCCCCGGTTTCCC
GGCGTTATCCCAGTCTTACAGGCAGGTTGCCCACGTGTTACTCACCCGTCCGC
CGCTAACCTTTTAAAAGCAAGCTTTTAAAAGGTCCGCACGACTTGCATGTATT
AGGCACGCCGCCAGCGTTCGTCCTGAGC

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacillus shackletonii strain LMG 18435 scaffold8, whole genome shotgun sequence	601	601	100%	9.00E-169	91.57%	NZ_LJJC01000011.1
Bacillus shackletonii strain LMG 18435 super19, whole genome shotgun sequence	601	2405	100%	9.00E-169	91.57%	NZ_LJJC01000006.1
Bacillus acidicola strain FJAT-2406 Scaffold1, whole genome shotgun sequence	601	5913	97%	9.00E-169	92.22%	NZ_KV440953.1
Bacillus methanolicus MGA3, complete genome	599	5322	96%	3.00E-168	92.27%	NZ_CP007739.1
Bacillus smithii strain DSM 4216, complete genome	597	6573	100%	1.00E-167	91.30%	NZ_CP012024.1
Bacillus shackletonii strain LMG 18435 super11, whole genome shotgun sequence	595	1787	100%	4.00E-167	91.34%	NZ_LJJC01000004.1
Quasibacillus thermotolerans strain SGZ-8 Contig10, whole genome shotgun sequence	582	582	96%	3.00E-163	91.53%	NZ_JWJE02000010.1
Edaphobacillus lindanitolerans strain MNA4, whole genome shotgun sequence	580	580	96%	1.00E-162	91.67%	NZ_FTPL01000008.1
Bacillus campisalis strain SA2-6 scf7180000001092, whole genome shotgun sequence	577	577	0.97	2E-161	0.9127	NZ_LAYY01000014.1
Bacillus marisflavi strain JCM 11544 Contig2, whole genome shotgun sequence	577	577	0.96	2E-161	0.9143	NZ_LGUE01000011.1 Bottom of Form

Isolate code: NT

UF1 sequence:
AGTCGTGCGGACCTTTTAAAGCTTGCTTTTAAAAGGTTAGCGGCGGACGGGT
GAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACGCCGGGAAACC
GGGGCTAATACCGGATAGTTTTTTCCTCCGCATGGAGGAAAAAGGAAAGGCG
GCTTCGGCTGCCACTTACAGATGGGCCCGCGGCGCATTAGCTAGTTGGCGGG
GTAACGGCCCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGG
CCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGG
GAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGAAG
AAGGCCTTCGGGTCGTAAAACTCTGTTGCCGGGGAAGAACAAGTGCCGTTCG
AACAGGGCGGCGCCTTGACGGTACCCGGCCAGAAAGCCACGGC

UR1 sequence:
GAGTTTTACGACCCGAAGGCCTTCTTCACTCACGCGGCGTTGCTCCGTCAGAC
TTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCC
GTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTT
GCCTTGGTGGGCCGTTACCCCGCCAACTAGCTAATGCGCCGCGGGCCCATCT
GTAAGTGGCAGCCGAAGCCGCCTTTCCTTTTTCCTCCATGCGGAGGAAAAAA
CTATCCGGTATTAGCCCCGGTTTCCCGGCGTTATCCCAGTCTTACAGGCAGGT
TGCCCACGTGTTACTCACCCGTCCGCCGCTAACCTTTTAAAAGCAAGCTTTTA
AAAGGTCCGCACGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTGA
GCCAATCAAAATTCAAGAAC

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacillus shackletonii strain LMG 18435 scaffold8, whole genome shotgun sequence	625	625	100%	6.00E-176	91.48%	NZ_LJJC01000011.1
Bacillus shackletonii strain LMG 18435 super19, whole genome shotgun sequence	625	2501	100%	6.00E-176	91.48%	NZ_LJJC01000006.1
Bacillus shackletonii strain LMG 18435 super11, whole genome shotgun sequence	619	1859	100%	3.00E-174	91.27%	NZ_LJJC01000004.1
Bacillus smithii strain DSM 4216, complete genome	604	6654	100%	8.00E-170	90.59%	NZ_CP012024.1
Bacillus acidicola strain FJAT-2406 Scaffold1, whole genome shotgun sequence	603	5935	92%	3.00E-169	92.42%	NZ_KV440953.1
Bacillus methanolicus MGA3, complete genome	590	5295	92%	2.00E-165	92.00%	NZ_CP007739.1
Quasibacillus thermotolerans strain SGZ-8 Contig10, whole genome shotgun	584	584	92%	1.00E-163	91.73%	NZ_JWJE02000010.1

sequence						
Bacillus campisalis strain SA2-6 scf7180000001092, whole genome shotgun sequence	579	579	92%	5.00E-162	91.47%	NZ_LAYY01000014.1
Bacillus marisflavi strain JCM 11544 Contig2, whole genome shotgun sequence	579	579	91%	5.00E-162	91.63%	NZ_LGUE01000011.1
Bacillus atrophaeus strain SRCM101359 chromosome, complete genome	579	4577	92%	5.00E-162	91.45%	NZ_CP021500.1

Isolate code: OT
UF1 sequence:
CCTGCCTGTACGACCGGGATAACTCCGGGAAACCGGAGCTAATACCGGATAG
GATGCCGAACCGCATGGTTCGGCATGGAAAGGCCTTTGAGCCGCGTACAGAT
GGGCCTGCGGCGCATTAGCTAGTTGGTGGGGTAACGGCCTACCAAGGCGACG
ATGCGTAGCCGACCTGAGAGGGTGAACGGCCACACTGGGACTGAGACACGG
CCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAA
GTCTGACGGAGCAACGCCGCGTGAGTGAGGAAGGTCTTCGGATCGTAAAACT
CTGTTGTCAGGGAAGAACCGCCGGGATGACCTCCCGGTCTGACGGTACCTG

UR1 sequence:
CTTCCTCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGAT
TCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGG
CCGTTCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTAGGCCGTTACCC
CACCAACTAGCTAATGCGCCGCAGGCCCATCTGTACGCGGCTCAAAGGCCTT
TCCATGCCGAACCATGCGGTTCGGCATCCTATCCGGTATTAGCTCCGGTTTCC
CGGAGTTATCCCGGTCGTACAGGCAGGTTGCCTACGTGTTACTCACCCGTCCG
CCGCTAACCTCAGGAATGCAAGCACTCCATCGGTTCGCTCGACTTGCATGTAT
TAGGCACGCCGCCAGCGTTCGTCCTGA

BLAST Output:

Description	**Max Score**	**Total Score**	**Query Cover**	**E value**	**Per. Ident**	**Accession**
Aneurinibacillus migulanus strain DSM 2895 super7, whole genome shotgun sequence	556	556	97%	2.00E-155	92.54%	NZ_LGUG01000004.1
Bacillus pumilus strain SH-B9, complete genome	525	4199	100%	5.00E-146	90.59%	NZ_CP011007.1
Bacillus amyloliquefaciens DSM 7 = ATCC 23350, complete sequence	525	5155	100%	5.00E-146	90.55%	NC_014551.1

Paenibacillus macerans strain 8244 scaffold1, whole genome shotgun sequence	523	2766	100%	2.00E-145	90.37%	NZ_KN125580.1
Bacillus weihaiensis strain Alg07 chromosome, complete genome	520	5194	100%	2.00E-144	90.30%	NZ_CP016020.1
Bacillus subtilis subsp. subtilis str. 168 complete genome	520	5098	100%	2.00E-144	90.30%	NC_000964.3
Bacillus licheniformis DSM 13 = ATCC 14580, complete sequence	520	3570	100%	2.00E-144	90.32%	NC_006270.3
Bacillus glycinifermentans isolate BGLY genome assembly, chromosome: 1	514	4088	100%	1.00E-142	90.07%	NZ_LT603683.1
Bacillus pseudofirmus OF4, complete sequence	514	3523	100%	1.00E-142	90.12%	NC_013791.2
Vibrio ostreicida strain UCD-KL16 scaffold_60, whole genome shotgun sequence	510	510	100%	1.00E-141	89.80%	NZ_MPHM01000060.1

Isolate code: PT
UF1 sequence:
TCCTGAGGTTAGCGGCGGACGGGTGAGTAACACGTAGGCAACCTGCCTGTAC
GACCGGGATAACTCCGGGAAACCGGAGCTAATACCGGATAGGATGCCGAAC
CGCATGGTTCGGCATGGAAAGGCCTTTGAGCCGCGTACAGATGGGCCTGCGG
CGCATTAGCTAGTTGGTGGGGTAACGGCCTACCAAGGCGACGATGCGTAGCC
GACCTGAGAGGGTGAACGGCCACACTGGGACTGAGACACGGCCCAGACTCC
TACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA
GCAACGCCGCGTGAGTGAGGAAGGTCTTCGGATCGTAAAACTCTGTTGTCAG
GGAAGAACCGCCGGGATGACCTCCCGGTCTGACGGTACCTGACGA

UR1 sequence:
ACCTTCCTCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGT
GGCCGTTCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTAGGCCGTTAC
CCCACCAACTAGCTAATGCGCCGCAGGCCCATCTGTACGCGGCTCAAAGGCC
TTTCCATGCCGAACCATGCGGTTCGGCATCCTATCCGGTATTAGCTCCGGTTT
CCCGGAGTTATCCCGGTCGTACAGGCAGGTTGCCTACGTGTTACTCACCCGTC
CGCCGCTAACCTCAGGAATGCAAGCACTCCATCGGTTCGCTCGACTTGCATGT
ATTAGGCACGCCGCCAGCGTTCGTCCTG

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Aneurinibacillus migulanus strain DSM 2895 super7, whole genome shotgun sequence	584	584	98%	9.00E-164	93.02%	NZ_LGUG01000004.1
Aneurinibacillus tyrosinisolvens strain LL-002, whole genome shotgun sequence	560	560	99%	1.00E-156	91.83%	NZ_BBWZ01000108.1
Bacillus indicus strain DSM 16189 Contig19, whole genome shotgun sequence	507	507	88%	2.00E-140	92.01%	NZ_JNVC02000019.1
Rubeoparvulum massiliense strain mt6, whole genome shotgun sequence	499	499	96%	3.00E-138	89.77%	NZ_CVPE01000004.1
Bacillus pumilus strain SH-B9, complete genome	490	3918	90%	2.00E-135	90.67%	NZ_CP011007.1
Bacillus amyloliquefaciens DSM 7 = ATCC 23350, complete sequence	490	4814	90%	2.00E-135	90.62%	NC_014551.1
Aeribacillus pallidus strain 8m3 NODE_1, whole genome shotgun sequence	488	488	89%	7.00E-135	90.91%	NZ_LWBR01000013.1
Bacillus glycinifermentans isolate BGLY genome assembly, chromosome: 1	484	3851	90%	9.00E-134	90.37%	NZ_LT603683.1
Bacillus subtilis subsp. subtilis str. 168 complete genome	484	4747	0.9	9E-134	0.9035	NC_000964.3
Bacillus licheniformis DSM 13 = ATCC 14580, complete sequence	484	3361	0.9	9E-134	0.9037	NC_006270.3

Isolate code: AM
UF1 sequence:
GGATCAGTGGCGAACGGGTGAGTAACACGTGAGCAATCTGCCCCTGACTCTG
GGATAAGCGCTGGAAACGGCGTCTAATACTGGATACGAGCTGCGAAGGCATC
TTCAGCAGCTGGAAAGAACTTCGGTCAGGGATGAGCTCGCGGCCTATCAGCT
TGTTGGTGAGGTAATGGCTCACCAAGGCGTCGACGGGTAGCCGGCCTGAGAG
GGTGACCGGCCACACTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCAACGCCGC
GTGAGGGACGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGGGAAGAAGCG
AAAGTGACGGTACCTGCAGAAAAAGCGCCGGC

UR1 sequence:
GCCGTCGTCCCTCACGCGGCGTTGCTGCATCAGGCTTTCGCCCATTGTGCAAT
ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGT
GGCCGGTCACCCTCTCAGGCCGGCTACCCGTCGACGCCTTGGTGAGCCATTA
CCTCACCAACAAGCTGATAGGCCGCGAGCTCATCCCTGACCGAAGTTCTTTCC
AGCTGCTGAAGATGCCTTCGCAGCTCGTATCCAGTATTAGACGCCGTTTCCAG
CGCTTATCCCAGAGTCAGGGGCAGATTGCTCACGTGTTACTCACCCGTTCGCC
ACTGATCCACCCAGCAAGCTGGGCTTCACCGTTCGACTTGCATGTGTTAAGCA
CGCCGCCAGCGTTCATCCTGA

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Microbacterium aurum strain KACC 15219 chromosome, complete genome	689	689	100%	0.00E+00	98.22%	NZ_CP018762.1
Microbacterium ginsengisoli strain DSM 18659 RR49_contig000074, whole genome shotgun sequence	673	673	100%	0.00E+00	97.46%	NZ_JYIY01000074.1
Microbacterium paludicola strain CC3, complete genome	673	1346	100%	0.00E+00	97.46%	NZ_CP018134.1
Microbacterium pygmaeum strain DSM 23142 genome assembly, chromosome: I	665	665	99%	0.00E+00	97.20%	NZ_LT629692.1
Microbacterium hominis NBRC 15708, whole genome shotgun sequence	656	656	100%	0.00E+00	96.71%	NZ_BCWI01000036.1
Agrococcus casei LMG 22410, whole genome shotgun sequence	645	645	100%	0.00E+00	96.19%	NZ_FUHU01000045.1
Microbacterium oleivorans NBRC 103075, whole genome shotgun sequence	645	645	100%	0.00E+00	96.20%	NZ_BCRG01000019.1
Agrococcus jejuensis strain DSM 22002 genome assembly, chromosome: I	645	1291	100%	0.00E+00	96.19%	NZ_LT629695.1
Microbacterium hydrocarbonoxydans strain SA35 RS84_contig000001, whole genome shotgun sequence	634	634	100%	8.00E-179	95.70%	NZ_JYJB01000001.1
Microbacterium chocolatum strain SIT 101 chromosome,	628	1257	100%	4.00E-	95.45%	NZ_CP015810.1

complete genome				177		

Isolate code: BM
UF1 sequence:
ACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCG
CATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGC
GTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAG
CCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTC
CTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGG
AGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTA
GGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCA
GAAAGCCACGGCTA

UR1 sequence:
AGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCAC
TCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTG
CTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACC
CTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTA
GCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAAT
TTCGAACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAG
TTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCT
AACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGC
ACGCCGCCAGCGTTCATCCTGAGC

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacillus thuringiensis YBT-1518, complete genome	697	10365	100%	0.00E+00	100.00%	NC_022873.1
[Bacillus thuringiensis] serovar konkukian str. 97-27 chromosome, complete genome	697	9745	100%	0.00E+00	100.00%	NC_005957.1
Bacillus anthracis str. Sterne chromosome, complete genome	697	7648	100%	0.00E+00	100.00%	NC_005945.1
Bacillus anthracis str. Ames chromosome, complete genome	697	7648	100%	0.00E+00	100.00%	NC_003997.3
Bacillus cereus ATCC 14579 chromosome, complete genome	697	9031	100%	0.00E+00	100.00%	NC_004722.1
Bacillus pseudomycoides DSM 12442 chromosome, whole genome shotgun	680	680	100%	0.00E+00	99.20%	NZ_CM000745.1

sequence						
Bacillus mycoides strain ATCC 6462 chromosome, complete genome	675	8053	100%	0.00E+00	98.94%	NZ_CP009692.1
Bacillus cytotoxicus NVH 391-98, complete genome	614	7890	100%	1.00E-172	96.02%	NC_009674.1
Bacillus halosaccharovorans strain DSM 25387 Scaffold3, whole genome shotgun sequence	575	575	0.99	5E-161	0.944	NZ_KV917373.1
Bacillus halosaccharovorans strain DSM 25387 Scaffold1, whole genome shotgun sequence	571	5649	0.99	6E-160	0.9415	NZ_KV917371.1

Isolate code: CM
UF1 sequence:
CCTGACTCTGGGATAACGCTGGAAACGGCGTCTAATACTGGATACAAGCTGC
GAAGGCATCTTCATCAGCTGGAAAGAATTTCGGTCAGGGATGAGCTCGCGGC
CTATCAGCTTGTTGGTGAGGTAACGGCTCACCAAGGCGTCGACGGGTAGCCG
GCCTGAAAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAAACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGC
AACACCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTTAGCATGG
AATAAGCGAAAGTGACGGTACCTGCATAAAAAGCGCCGGCTCAG

UR1 sequence:
ACGCGGCGTTGCTGCATCAGGCTTTCGCCCATTGTGCAATATTCCCCACTGCT
GCCTCCCGTAGGAGTCTGGGCCGTGTCTCAATCCCAGTGTGGCCGGTCACCCT
CTCAGGCCGGCTACCCGTCGACGCCTTGGTGAGCCGTTACCTCACCAACAAG
CTGATAGGCCGCGAGCTCATCCCTGACCGAAATTCTTTCCAGCTGCTGAAGAT
GCCTTCGCAGCTCGTATCCAGTATTAGACGCCGTTTCCAGCGCTTATCCCAGA
GTCAGGGGCAGATTGCTCACGTGTTACTCACCCGTTCGCCACTGATCCAGCA
GAGCAAGCTCCACCTTCACCGTTCGACTTGCATGTGTTAAGCACGCCGCCAG
CGTTCATCCTGAGC

BLAST Output:

Description	**Max Score**	**Total Score**	**Query Cover**	**E value**	**Per. Ident**	**Accession**
Microbacterium aurum strain KACC 15219 chromosome, complete genome	564	564	0.98	1E-157	0.9573	NZ_CP018762.1
Microbacterium ginsengisoli strain DSM 18659 RR49_contig000074, whole genome shotgun sequence	562	562	0.99	4E-157	0.9547	NZ_JYIY01000074.1

Microbacterium paludicola strain CC3, complete genome	556	1113	0.99	2E-155	0.9518	NZ_CP018134.1
Microbacterium hominis NBRC 15708, whole genome shotgun sequence	542	542	0.99	5E-151	0.9435	NZ_BCWI01000036.1
Microbacterium oleivorans NBRC 103075, whole genome shotgun sequence	536	536	0.99	2E-149	0.9407	NZ_BCRG01000019.1
Microbacterium pygmaeum strain DSM 23142 genome assembly, chromosome: I	536	536	0.98	2E-149	0.943	NZ_LT629692.1
Microbacterium chocolatum strain SIT 101 chromosome, complete genome	531	1062	0.99	1E-147	0.938	NZ_CP015810.1
Agrococcus casei LMG 22410, whole genome shotgun sequence	529	529	0.99	4E-147	0.9377	NZ_FUHU01000045.1
Microbacterium hydrocarbonoxydans strain SA35 RS84_contig000001, whole genome shotgun sequence	525	525	0.99	5E-146	0.935	NZ_JYJB01000001.1
Agrococcus jejuensis strain DSM 22002 genome assembly, chromosome: I	523	1047	0.99	2E-145	0.9348	NZ_LT629695.1

Isolate code: EM
UF1 sequence:
TAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGAT
AACTCCGGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGGTTC
AAGGATGAAAGACGGTTTCGGCTGTCACTTACAGATGGACCCGCGGCGCATT
AGCTAGTTGGTGGGGTAATGGCTCACCAAGGCGACGATGCGTAGCCGACCTG
AGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG
CCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGA
ACAAGTGCGAGAGTAACTGCTCGCACCTTGACGGTACCTA

UR1 sequence:
AAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGG
AAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAG
TGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCAT
TACCCCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGACAGCCGA
AACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCT

CCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTC
ACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTG
CATGTATTAGGCACGCCGCCAGCGTTCGTCCT

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacillus pumilus strain SH-B9, complete genome	743	5942	100%	0.00E+00	100.00%	NZ_CP011007.1
Bacillus indicus strain DSM 16189 Contig19, whole genome shotgun sequence	623	623	99%	2.00E-175	94.76%	NZ_JNVC02000019.1
Bacillus halosaccharovorans strain DSM 25387 Scaffold3, whole genome shotgun sequence	610	610	100%	1.00E-171	94.03%	NZ_KV917373.1
Bacillus halosaccharovorans strain DSM 25387 Scaffold1, whole genome shotgun sequence	610	6082	100%	1.00E-171	94.03%	NZ_KV917371.1
Bacillus tuaregi strain Marseille-P2489T, whole genome shotgun sequence	610	3876	100%	1.00E-171	94.03%	NZ_LT629731.1
Quasibacillus thermotolerans strain SGZ-8 Contig10, whole genome shotgun sequence	606	606	99%	2.00E-170	94.01%	NZ_JWJE02000010.1
Bacillus subtilis subsp. subtilis str. 168 complete genome	606	5972	1	2E-170	0.9622	NC_000964.3
Bacillus koreensis strain DSM 16467 Contig9, whole genome shotgun sequence	604	604	1	7E-170	0.9384	NZ_LILC01000014.1
Bacillus atrophaeus strain SRCM101359 chromosome, complete genome	604	4823	1	7E-170	0.9381	NZ_CP021500.1
Bacillus amyloliquefaciens DSM 7 = ATCC 23350, complete sequence	604	6027	1	7E-170	0.9381	NC_014551.1

Isolate code: FM
UF1 sequence:
GGCGGACGGGTGAGTAACATTTAGGAATCTGCCTAGTAGTGGGGGATAGCTC
GGGGAAACTCGAATTAATACCGCATACGACCTACGGGTGAAAGGGGGCGCA
AGCTCTTGCTATTAGATGAGCCTAAATCAGATTAGCTAGTTGGTGGGGTAAA

GGCCCACCAAGGCGACGATCTGTAACTGGTCTGAGAGGATGATCAGTCACAC
CGGAACTGAGACACGGTCCGGACTCCTACGGGAGGCAGCAGTGGGGAATATT
GGACAATGGGGGCAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCT
TTTGGTTGTAAAGCACTTTAAGCAGGGAGGAGAGGCTAATGGTTAATACCCA
TTAGATTAGACGTTACCTGCAGAATAAGCACCGGC

UR1 sequence:
ACGGGTGAGTAACATTTAGGAATCTGCCTAGTAGTGGGGGATAGCTCGGGGA
AACTCGAATTAATACCGCATACGACCTACGGGTGAAAGGGGGCGCAAGCTCT
TGCTATTAGATGAGCCTAAATCAGATTAGCTAGTTGGTGGGGTAAAGGCCCA
CCAAGGCGACGATCTGTAACTGGTCTGAGAGGATGATCAGTCACACCGGAAC
TGAGACACGGTCCGGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACA
ATGGGGGCAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCTTTTGG
TTGTAAAGCACTTTAAGCAGGGAGGAGAGGCTAATGGTTAATACCCATTAGA
TTAGACGTTACCTGCAGAATAAGCACCG

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Moraxella osloensis strain CCUG 350, complete genome	730	2922	100%	0.00E+00	99.75%	NZ_CP014234.1
Moraxella porci DSM 25326 strain CCUG 54912 54912T_ctg_0000016, whole genome shotgun sequence	569	569	100%	2.00E-159	92.46%	NZ_MUYV01000016.1
Moraxella lincolnii strain CCUG 9405 CCUG9405T_R1__paired__trimmed__paired__contig_21, whole genome shotgun sequence	564	564	100%	1.00E-157	92.23%	NZ_MUYT01000021.1
Psychrobacter arcticus 273-4, complete genome	540	2161	100%	2.00E-150	91.11%	NC_007204.1
Psychrobacter urativorans strain R310.10B chromosome, complete genome	540	3242	100%	2.00E-150	91.11%	NZ_CP012678.1
Psychrobacter alimentarius strain PAMC 27889 chromosome, complete genome	534	2674	100%	9.00E-149	90.84%	NZ_CP014945.1
Pseudohongiella acticola strain KCTC 42131 KCTC42131_S7, whole	520	520	100%	2.00E-144	90.17%	NZ_MASR01000007.1

genome shotgun sequence						
Pseudohongiella acticola strain KCTC 42131 KCTC42131_S6, whole genome shotgun sequence	520	520	100%	2.00E-144	90.17%	NZ_MASR01000006.1
Pseudohongiella acticola strain KCTC 42131 KCTC42131_S3, whole genome shotgun sequence	520	520	1	2E-144	0.9017	NZ_MASR01000003.1
Moraxella catarrhalis BBH18, complete genome	520	2080	1	2E-144	0.9023	NC_014147.1
Psychrobacter arcticus 273-4, complete genome	540	2161	100%	2.00E-150	91.11%	NC_007204.1

Isolate code: GM
UF1 sequence:
ACACGTGAGCAATCTGCCCCTGACTCTGGGATAAGCGCTGGAAACGGCGTCT
AATACTGGATACGAGCTGCGAAGGCATCTTCAGCAGCTGGAAAGAACTTCGG
TCAGGGATGAGCTCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCA
AGGCGTCGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTG
AGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT
GGGCGAAAGCCTGATGCAGCAACGCCGCGTGAGGGACGACGGCCTTCGGGT
TGTAAACCTCTTTTAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAAA
AGCGCCGG

UR1 sequence:
CTGCATCAGGCTTTCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAG
GAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGGTCACCCTCTCAGGCCGG
CTACCCGTCGACGCCTTGGTGAGCCATTACCTCACCAACAAGCTGATAGGCC
GCGAGCTCATCCCTGACCGAAGTTCTTTCCAGCTGCTGAAGATGCCTTCGCAG
CTCGTATCCAGTATTAGACGCCGTTTCCAGCGCTTATCCCAGAGTCAGGGGCA
GATTGCTCACGTGTTACTCACCCGTTCGCCACTGATCCACCCAGCAAGCTGGG
CTTCACCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCATCCTGAGC
CAGGAT

BLAST Output:

Description	**Max Score**	**Total Score**	**Query Cover**	**E value**	**Per. Ident**	**Accession**
Microbacterium aurum strain KACC 15219 chromosome, complete genome	634	634	100%	8.00E-179	97.08%	NZ_CP018762.1
Microbacterium hominis NBRC 15708, whole genome shotgun sequence	632	632	100%	3.00E-178	97.07%	NZ_BCWI01000036.1

Microbacterium paludicola strain CC3, complete genome	627	1254	100%	1.00E-176	96.80%	NZ_CP018134.1
Microbacterium ginsengisoli strain DSM 18659 RR49_contig000074, whole genome shotgun sequence	616	616	100%	3.00E-173	96.27%	NZ_JYIY01000074.1
Agrococcus casei LMG 22410, whole genome shotgun sequence	606	606	100%	2.00E-170	95.76%	NZ_FUHU01000045.1
Microbacterium oleivorans NBRC 103075, whole genome shotgun sequence	599	599	100%	3.00E-168	95.48%	NZ_BCRG01000019.1
Microbacterium pygmaeum strain DSM 23142 genome assembly, chromosome: I	599	599	100%	3.00E-168	95.47%	NZ_LT629692.1
Microbacterium chocolatum strain SIT 101 chromosome, complete genome	593	1187	100%	1.00E-166	95.23%	NZ_CP015810.1
Agrococcus jejuensis strain DSM 22002 genome assembly, chromosome: I	584	1169	100%	8.00E-164	94.69%	NZ_LT629695.1
Microbacterium ketosireducens strain DSM 12510 RS81_contig000009, whole genome shotgun sequence	582	582	100%	3.00E-163	94.68%	NZ_JYIZ01000009.1

Isolate code: HM
UF1 sequence:
TAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGAT
AACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTC
GAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATT
AGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTG
AGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG
CCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGA
ACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCC
ACGGC

UR1 sequence:
GAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGAC
TTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCC
GTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTT
GCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACGCGGGTCCATCC

ATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATG
TTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGT
TACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTA
ATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTC

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacillus thuringiensis YBT-1518, complete genome	774	11528	100%	0.00E+00	100.00%	NC_022873.1
[Bacillus thuringiensis] serovar konkukian str. 97-27 chromosome, complete genome	774	10831	100%	0.00E+00	100.00%	NC_005957.1
Bacillus anthracis str. Sterne chromosome, complete genome	774	8501	100%	0.00E+00	100.00%	NC_005945.1
Bacillus anthracis str. Ames chromosome, complete genome	774	8501	100%	0.00E+00	100.00%	NC_003997.3
Bacillus cereus ATCC 14579 chromosome, complete genome	774	10040	100%	0.00E+00	100.00%	NC_004722.1
Bacillus pseudomycoides DSM 12442 chromosome, whole genome shotgun sequence	758	758	100%	0.00E+00	99.28%	NZ_CM000745.1
Bacillus mycoides strain ATCC 6462 chromosome, complete genome	747	8912	100%	0.00E+00	98.81%	NZ_CP009692.1
Bacillus cytotoxicus NVH 391-98, complete genome	691	8893	100%	0.00E+00	96.42%	NC_009674.1
Bacillus halosaccharovorans strain DSM 25387 Scaffold3, whole genome shotgun sequence	640	640	100%	2.00E-180	94.27%	NZ_KV917373.1
Bacillus halosaccharovorans strain DSM 25387 Scaffold1, whole genome shotgun sequence	636	6295	1	2E-179	0.9405	NZ_KV917371.1

Isolate code: JM
UF1 sequence:
GGTGCGGAACACGTGTGCAACCTGCCTTTATCAGGGGGATAGCCTTTCGAAA

GGAAGATTAATACCCCATAATATATTAATTGGCATCAATTGATATTGAAAACT
ACGGTGGATAGAGATGGGCACGCGCAAGATTAGATAGTTGGTAGGGTAACG
GCCTACCAAGTCAGTGATCTTTAGGGGGCCTGAGAGGGTGATCCCCCACACT
GGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATT
GGACAATGGGTTAGCGCCTGATCCAGCCATCCCGCGTGAAGGACGACGGCCC
TATGGGTTGTAAACTTCTTTTGTATAGGGATAAACCTACTCTCGTGAGAGTAG
CTGAAGGTACTATACGAATAAGCACCGGCT

UR1 sequence:
ATAGGGCCGTCGTCCTTCACGCGGGATGGCTGGATCAGGCGCTAACCCATTG
TCCAATATTCCTCACTGCTGCCTCCCGTAGGAGTCTGGTCCGTGTCTCAGTAC
CAGTGTGGGGGATCACCCTCTCAGGCCCCCTAAAGATCACTGACTTGGTAGG
CCGTTACCCTACCAACTATCTAATCTTGCGCGTGCCCATCTCTATCCACCGTA
GTTTTCAATATCAATTGATGCCAATTAATATATTATGGGGTATTAATCTTCCTT
TCGAAAGGCTATCCCCCTGATAAAGGCAGGTTGCACACGTGTTCCGCACCCG
TACGCCGCTCTCTCTGTCCCGAAAGACAAATACCGCTCGGCTTGCATGTGTTA
GGCCTCCCGCTAGCGTTCATCCTGAGC

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Chryseobacterium scophthalmum strain DSM 16779, whole genome shotgun sequence	689	689	100%	0.00E+00	98.22%	NZ_FSRQ01000008.1
Chryseobacterium hominis strain DSM 19326, whole genome shotgun sequence	673	673	100%	0.00E+00	97.46%	NZ_FNWX01000073.1
Chryseobacterium vrystaatense strain LMG 22846 contig06, whole genome shotgun sequence	645	645	100%	0.00E+00	96.19%	NZ_JPRI01000006.1
Chryseobacterium formosense strain LMG 24722 contig04, whole genome shotgun sequence	640	640	100%	2.00E-180	95.96%	NZ_JPRP01000004.1
Chryseobacterium daeguense DSM 19388 H560DRAFT_scaffold00025.25_C, whole genome shotgun sequence	628	628	100%	4.00E-177	95.43%	NZ_AUMT01000026.1
Chryseobacterium zeae strain DSM 27623, whole genome shotgun sequence	628	628	100%	4.00E-177	95.45%	NZ_FSRK01000004.1
Chryseobacterium taihuense strain CGMCC 1.10941,	623	623	100%	2.00E-175	95.20%	NZ_FNHD01000030.1

whole genome shotgun sequence						
Chryseobacterium soli strain DSM 19298 Contig01, whole genome shotgun sequence	623	623	100%	2.00E-175	95.18%	NZ_JPRH01000001.1
Riemerella columbipharyngis strain DSM 24015, whole genome shotgun sequence	617	617	100%	8.00E-174	94.92%	NZ_FNAS01000031.1
Chryseobacterium piscicola strain DSM 21068, whole genome shotgun sequence	617	617	1	8E-174	0.9492	NZ_FTOJ01000019.1

Isolate code: LM
UF1 sequence:
TCGAGCGGGGAAGAGTAGCTTGCTACTTAACCTAGCGGCGGACGGGTGAGTA
ATGCTTAGGAATCTGCCTATTAGTGGGGGACAACATCTCGAAAGGGATGCTA
ATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTA
ATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAG
GCGACGATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGA
CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGG
GGGAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCTTTTGGTTGTA
AAGCACTTTAAGCGAGGAGGAGGCTACCGAGATTAATACTCTTGGATAGTGG
ACGTTACTCGCAGAATAAG

UR1 sequence:
AGCCTCCTCCTCGCTTAAAGTGCTTTACAACCAAAAGGCCTTCTTCACACACG
CGGCATGGCTGGATCAGGGTTCCCCCCATTGTCCAATATTCCCCACTGCTGCC
TCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTC
AGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTA
ATCCGACTTAGGCTCATCTATTAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTC
CCGTAGGACGTATGCGGTATTAGCATCCCTTTCGAGATGTTGTCCCCCACTAA
TAGGCAGATTCCTAAGCATTACTCACCCGTCCGCCGCTAGGTTAAGTAGCAA
GCTACTCTTCCCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCA

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Acinetobacter beijerinckii CIP 110307 acLZq-supercont1.3, whole genome shotgun sequence	743	3711	100%	0.00E+00	98.57%	NZ_KB849765.1
Acinetobacter baumannii strain AB30 chromosome,	737	4427	100%	0.00E+00	98.34%	NZ_CP009257.1

complete genome						
Acinetobacter harbinensis strain HITLi 7 Scaffold1, whole genome shotgun sequence	734	2202	100%	0.00E+00	98.10%	NZ_JXBK01000001.1
Acinetobacter kookii strain ANC 4667, whole genome shotgun sequence	721	721	100%	0.00E+00	97.62%	NZ_FMYO01000019.1
Acinetobacter ursingii DSM 16037 = CIP 107286 acLZr-supercont1.1, whole genome shotgun sequence	706	706	99%	0.00E+00	97.14%	NZ_KB849710.1
Acinetobacter ursingii DSM 16037 = CIP 107286 acLZr-supercont1.9, whole genome shotgun sequence	706	706	99%	0.00E+00	97.14%	NZ_KB849719.1
Acinetobacter ursingii DSM 16037 = CIP 107286 acLZr-supercont1.10, whole genome shotgun sequence	706	1413	99%	0.00E+00	97.14%	NZ_KB849711.1
Acinetobacter ursingii DSM 16037 = CIP 107286 acLZr-supercont1.7, whole genome shotgun sequence	706	706	99%	0.00E+00	97.14%	NZ_KB849717.1
Acinetobacter gyllenbergii NIPH 230 adfcq-supercont1.2, whole genome shotgun sequence	704	2114	1	0	0.969	NZ_KI530704.1
Acinetobacter radioresistens DSM 6976 = NBRC 102413 = CIP 103788 acLrZ-supercont1.7, whole genome shotgun sequence	699	699	1	0	0.9667	NZ_KB849747.1

Isolate code: KM
UF1 sequence:
CGGAGTTAGTGGCGGACGGGTGAGTAACACGTGGGAACGTGCCTTTAGGTTC
GGAATAACTCAGGGAAACTTGTGCTAATACCGAATGTGCCCTTCGGGGGAAA
GATTTATCGCCTTTAGAGCGGCCCGCGTCTGATTAGCTAGTTGGTGAGGTAAA
GGCTCACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACAT
TGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATCTT
GCGCAATGGGCGAAAGCCTGACGCAGCCATGCCGCGTGAATGATGAAGGTCT
TAGGATTGTAAAATTCTTTCACCGGGGACGATAATGACGGTACCCGGAGAAG
AAGCCCC

UR1 sequence:
TTTACAATCCTAAGACCTTCATCATTCACGCGGCATGGCTGCGTCAGGCTTTC
GCCCATTGCGCAAGATTCCCCACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGT
CTCAGTCCCAATGTGGCTGATCATCCTCTCAGACCAGCTACTGATCGTCGCCT
TGGTGAGCCTTTACCTCACCAACTAGCTAATCAGACGCGGGCCGCTCTAAAG
GCGATAAATCTTTCCCCCGAAGGGCACATTCGGTATTAGCACAAGTTTCCCTG
AGTTATTCCGAACCTAAAGGCACGTTCCCACGTGTTACTCACCCGTCCGCCAC
TAACTCCGAAGAGTTCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTC
GCTCTGAGCCAGGATCAAAC

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Brevundimonas vesicularis strain FDAARGOS_289 chromosome, complete genome	688	1376	100%	0.00E+00	100.00 %	NZ_CP022048.2
Brevundimonas naejangsanensis strain B1 chromosome, complete genome	654	1309	100%	0.00E+00	98.39%	NZ_CP015614.1
Brevundimonas aveniformis DSM 17977 G391DRAFT_scaffold00001.1_C, whole genome shotgun sequence	643	643	100%	0.00E+00	97.85%	NZ_AUAO01000001.1
Caulobacter vibrioides strain T5M6 contig_129, whole genome shotgun sequence	640	640	99%	2.00E-180	97.84%	NZ_LNIY01000034.1
Caulobacter segnis ATCC 21756, complete genome	640	1280	99%	2.00E-180	97.84%	NC_014100.1
Caulobacter crescentus NA1000, complete genome	640	1280	99%	2.00E-180	97.84%	NC_011916.1
Caulobacter crescentus CB15 chromosome, complete genome	640	1280	99%	2.00E-180	97.84%	NC_002696.2
Brevundimonas abyssalis TAR-001, whole genome shotgun sequence	638	638	1	6E-180	0.9758	NZ_BATC01000012.1
Brevundimonas viscosa strain CGMCC 1.10683, whole genome shotgun sequence	627	627	1	1E-176	0.9704	NZ_FOZV01000005.1
Brevundimonas bacteroides DSM 4726	616	616	1	3E-173	0.9651	NZ_JNIX01000007.1

Q333DRAFT_scaffold00001.1_C, whole genome shotgun sequence						

Isolate code: MM
UF1 sequence:
AGGTAACCTGCCTACTAGCGGGGGATAACTATTGGAAACGATAGCTAATACC
GCATAACAGCATTTAACCCATGTTAGATGCTTGAAAGGAGCAATTCGCTTCA
CTAGTAGATGGACCTGCGTTGTATTAGCTAGTTGGTGAGGTAACGGCTCACC
AAGGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACT
GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAA
TGGGGGCAACCCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGAT
CGTAAAGCTCTGTTGTAAGAGAAGAACGTGTGTGAGAGTGGAAAGTTCACAC
AGTGACGGTAACTTACCAGAAAGGGACGG

UR1 sequence:
TTACAACAGAGCTTTACGATCCGAAAACCTTCTTCACTCACGCGGCGTTGCTC
GGTCAGGGTTGCCCCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGGAG
TCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTAT
GTATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATACAACGCAG
GTCCATCTACTAGTGAAGCAATTGCTCCTTTCAAGCATCTAACATGGGTTAAA
TGCTGTTATGCGGTATTAGCTATCGTTTCCAATAGTTATCCCCCGCTAGTAGG
CAGGTTACCTACGCGTTACTCACCCGTTCGCAACTCTTCCAACTTTAGCAAGC
TAAAGTCTTCAGCGTTCTACTTGCATGTATTAGGCACGCCGCCAGCGTTCGT

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Streptococcus equinus strain AG46 BV58DRAFT_scf7180000000002_quiver_dupTrim_6839.1_C, whole genome shotgun sequence	710	4971	100%	0.00E+00	99.49%	NZ_JNLO01000001.1
Streptococcus gallolyticus subsp. gallolyticus DSM 16831, complete genome	660	3962	100%	0.00E+00	97.19%	NZ_CP018822.1
Streptococcus henryi DSM 19005 F601DRAFT_scaffold00033.33_C, whole genome shotgun sequence	610	610	100%	1.00E-171	94.91%	NZ_AQYA01000005.1
Streptococcus suis BM407 chromosome, complete genome	604	2419	100%	6.00E-170	94.66%	NC_012926.1

Streptococcus porci DSM 23759 G576DRAFT_scaffold00029.29_C, whole genome shotgun sequence	588	588	100%	6.00E-165	93.86%	NZ_AUIP01000031.1
Streptococcus orisratti DSM 15617 A3I7DRAFT_scaffold_69.70, whole genome shotgun sequence	588	588	100%	6.00E-165	93.88%	NZ_KB904514.1
Streptococcus ratti FA-1 = DSM 20564 strain FA-1 contig1, whole genome shotgun sequence	588	588	100%	6.00E-165	93.86%	NZ_AJTZ01000001.1
Streptococcus sanguinis SK36 chromosome, complete genome	588	2347	1	6E-165	0.9388	NC_009009.1
Streptococcus merionis DSM 19192 A3I5DRAFT_scaffold_23.24, whole genome shotgun sequence	577	577	1	1E-161	0.9335	NZ_KB904554.1
Streptococcus varani strain FF10, whole genome shotgun sequence	577	577	1	1E-161	0.9335	NZ_CTEN01000001.1

Isolate code: OM
UF1 sequence:
GCTTGCTCCCTGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCC
TATGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCT
ACGGGAGAAAGTGGGGGATCTTCGGACCTCGCGCTATCAGATGAGCCTAGGT
CGGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCCGTAACT
GGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAG
CCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGG
GAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATA
AGCACCGG

UR1 sequence:
TAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTC
TTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCC
CACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGA
TCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCTTTACCTCACC
AACTAGCTAATCCGACCTAGGCTCATCTGATAGCGTGAGGTCCGAAGATCCC
CCGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGT

CCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTGAATC
ATGGAGCAAGCTCCACTCATCCGCTCGACTTGC

BLAST Output:

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Pseudomonas stutzeri, complete sequence	739	2948	100%	0.00E+00	99.75%	NC_015740.1
Pseudomonas benzenivorans strain DSM 8628, whole genome shotgun sequence	712	712	100%	0.00E+00	98.51%	NZ_FNCT01000040.1
Pseudomonas alcaligenes strain NEB 585, complete genome	701	2047	100%	0.00E+00	98.01%	NZ_CP014784.1
Pseudomonas kuykendallii strain NRRL B-59562, whole genome shotgun sequence	689	689	100%	0.00E+00	97.52%	NZ_FNNU01000014.1
Pseudomonas putida KT2440 chromosome, complete genome	689	4829	100%	0.00E+00	97.52%	NC_002947.4
Pseudomonas citronellolis strain SJTE-3 chromosome, complete genome	684	3394	100%	0.00E+00	97.27%	NZ_CP015878.1
Pseudomonas fuscovaginae strain LMG 2158 genome assembly, chromosome: I	682	4045	99%	0.00E+00	97.49%	NZ_LT629972.1
Pseudomonas stutzeri strain 28a24 chromosome, complete genome	682	2719	99%	0.00E+00	97.49%	NZ_CP007441.1
Pseudomonas nitroreducens NBRC 12694, whole genome shotgun sequence	678	678	100%	0.00E+00	97.02%	NZ_BDAI01000032.1
Pseudomonas rhizosphaerae strain DSM 16299 chromosome, complete genome	678	4045	0.99	0	0.9726	NZ_CP009533.1