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MICROBIOLOGY

Evaluation of the motility and capacity of biofilm production by *Pseudomonas fluorescens* strains in residual milk

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Abstract: *Pseudomonas fluorescens* is known to have the ability to adhere and produce biofilm. The formation of biofilms is enhanced by cellular motility, particularly when mediated by flagella. Biofilm formed on surfaces such as those used for food production act as points of contamination, releasing pathogenic or deteriorating microorganisms and compromising the quality of products. We assessed two strains of *Pseudomonas fluorescens* PL5.4 and PL7.1, sampled from raw, chilled, buffalo milk, which was obtained from a dairy farm. Twitching and swarming motility assays were performed, in addition to the biofilm production evaluations at a temperature of 7 °C. Regarding the motility assays, only the PL5.4 strain scored positive for the swarming assay. On microplates, both strains presented themselves as strong biofilm producers at 7 °C. The PL5.4 strain was also able to form biofilm on a stainless steel structure and maintain this structure for up to 72 hours at refrigeration. The *Pseudomonas fluorescens* PL5.4 isolate was identified on the basis of a 99% sequence identity with *Pseudomonas fluorescens* A506, a strain used as a biocontrol in agriculture. Biofilm-forming bacteria, when adapted to low temperatures, become a constant source of contamination, damaging the production, quality, safety and shelf-life of products.

Key words: biofilm, buffalo milk, motility, *Pseudomonas fluorescens,* swarming, twitching.

INTRODUCTION

Buffalo milk has been growing in demand as raw material for the production of dairy products (Bailone et al. 2017, Gawande et al. 2022). Freshly collected milk has a low number of microorganisms and does not present health risks. However, the contamination of raw milk by microorganisms can originate from the animal itself, from human handlers or from the environment (Nucera et al. 2016). The temperature and conservation prior to pasteurization will determine the subsequent development of contaminating microbiota (Bailone et al. 2017). Cooling raw milk as a control strategy against the growth of mesophilic microorganisms coincidentally leads to the creation of the selective conditions under which psychrotrophic bacteria can still thrive (Puga et al. 2016, Ali et al. 2021, Parmar et al. 2022)

Pseudomonas spp. represents a problem both in primary production and during the processing of milk. They may cause mastitis and changes in dairy products, even if they are kept under strict refrigeration temperatures (Nucera et al. 2016). The *Pseudomonas* sp. are mobile gram-negative bacilli, consisting of one or more polar flagella, are aerobic, mesophilic and psycho-tolerant bacteria that are widely present in nature (Rossi et al. 2016, Nishimura et al. 2017). Among the *Pseudomonas* sp. isolated from

raw milk, *Pseudomonas fluorescens* is most often found (Puga et al. 2016, Rossi et al. 2016, Nishimura et al. 2017, Xu et al. 2017).

P. fluorescens is known to have adhesion and biofilm producing abilities (Rossi et al. 2016, Puga et al. 2016, Gallique et al. 2017). The ability to adhere to solid surfaces and the subsequent formation of an organized bacterial community are important steps in the establishment of biofilms (Gallique et al. 2017). The production of biofilms by *P. fluorescens* is enhanced by cell motility, particularly when mediated by flagella (Rossi et al. 2016, Gallique et al. 2017). The transition between the planktonic and the sessile state is often described as a cycle of regulated events that allows bacterial populations to adapt to growth on a given surface (Gallique et al. 2017). This transition is accompanied by changes in flagellar function, the production of type IV pili (T4P), the intracellular accumulation of the second messenger cyclic diguanylate (c-di-GMP) and the production of adhesins and exopolysaccharides that contribute to the formation of a community of organized biofilm (Gallique et al. 2017). The formation of biofilms on surfaces used for food production has been increasingly studied, because biofilms act as contamination points which release cells from pathogenic or deteriorating microorganisms, thereby compromising the quality of the raw material and its derivatives (Li et al. 2023). The scarcity of studies on biofilm formation by *P. fluorescens* in the production of milk and milk derivatives underscores the importance of this study (Lin et al. 2016, Rossi et al. 2016). In this sense, we sought to investigate the factors that predispose raw, chilled buffalo milk to the formation of biofilms by isolates found in refrigeration tanks.

MATERIALS AND METHODS

Bacterial isolates and culture conditions

Pseudomonas fluorescens PL5.4 and *Pseudomonas fluorescens* PL7.1 were used in this study and isolated from raw buffalo milk, which was obtained from a cooling tank on a dairy farm located in the municipality of Glorinha, Rio Grande do Sul, Brazil. They were obtained between 2012 and 2013 and had been isolated and identified by polymerase chain reaction (PCR) in a previous study. The strains were frozen in skim milk and stored at a temperature of -20 °C. They were subsequently thawed and seeded in Tryptic Soy Broth (TSB) and incubated at a temperature of 30°C for 48 h. After growth, the bacterial cultures were streak-plated on Trypticase Soy Agar plates (TSA) and incubated at 30°C for 48 h.

Evaluation of bacterial by twitching motility assay

The assay associated with twitching motility is related to the repetitive stretching and retracting movements of type IV pili structures which is observed on solid surfaces, interfaces or media with moderate viscosities (1% agar). *Pseudomonas fluorescens* strains PL5.4 and PL7.1 on Luria-Bertani agar (LB) 1% were stab inoculated to the base of the plate. After incubation at 30 °C for 24 h, the agar was removed and the motility zone was determined in mm by staining the petri dish with 2% crystal violet for 2 h. Each strain was tested in quadruplicate. The *P. aeruginosa* strain ATCC 27853 was used as a positive control (Otton et al. 2017).

Evaluation of bacterial by the swarming motility assay

The test associated with swarming motility evaluates for a dendritic pattern of movement and this is observed in the presence of specific

sources of nitrogen and carbon, as well as on less viscous surfaces, such as 0.4-0.7% agar. The assay was performed according to Otton et al. (2017) with some adaptations. Luria Bertani agar 0.5% supplemented with 12 mM glucose and 12 mM glutamate was used. Plates were dried for 1 hour under laminar flow and after this *Pseudomonas fluorescens* strains PL7.1 and PL5.4 were stab inoculated on the agar, up to the base of the plate. Incubation was performed in a humid atmosphere at 30 °C for 24 hours and at room temperature for a further 48 h and the motility zone was measured in mm. *Trypticase Soy Agar* (TSA) plates inoculated with the strains were used as negative controls. Each strain was tested in duplicate. The *P. aeruginosa* strain ATCC 27853 was used as the positive control. Test positivity was based on an 80% difference in halo size in the correlation between the Luria Bertani 0.5% test and the TSA.

Violet crystal assay for evaluation of biofilm formation in the presence of residual milk

The method described by Stepanovic et al. (2000) was used. To simulate the presence of milk on a surface, the microtiter plates were filled with 200 µL of skimmed UHT (*ultra high temperature*) bovine milk and, after two hours at room temperature, they were aspirated. The microtiter plate remained at room temperature for 2 h prior to the biofilm formation assay. After the growth of the bacterial strains of *Pseudomonas fluorescens* PL5.4 and PL7.1 on TSA plates, the colonies were resuspended in a 0.85% saline solution and turbidity was standardized at 0.5, according to the McFarland scale (1,5 x 10^8 CFU/mL).

The assay was performed in 96-well microtiter plates of polystyrene material. The strains were individually inoculated in octoplicates. 180 µL TSB (Himedia, India) were added to the wells, plus 0.25% glucose and 20 µL of bacterial suspension of each one of the individual strains. Wells with the negative control were inoculated under the same conditions, but without the addition of the strains. For the positive control, a strong biofilm-forming culture of *Staphylococcus epidermidis* ATCC 35984 (Moura et al. 2015) was used. The plates were incubated at 7 °C for 72 h, 23 °C for 24 h and at 37 °C for 24 h. After incubation, the wells were aspirated and the samples washed three times with 200 µL of 0.85% saline solution. Bacteria were fixed using 200 µL of methanol (PA) for 20 minutes and then aspirated and the microplates were inverted and allowed to dry overnight at room temperature. The staining procedure was performed with 200 µL of 0.5% crystal violet solution for 15 minutes, followed by a washing of the plates with sterile distilled water. After drying, the bacterial cells fixed and stained at the bottom of the wells were resuspended in 200 µL of 95% ethanol for 30 minutes and then quantification of the biofilm was carried out. The optical density (OD) of the bacterial biofilm was quantified with the aid of a microplate spectrophotometer reader to a wavelength of 570 nm (Type Anthos 2010 type 17 550 S. N° 4894). All strains were separated into categories using the OD of the bacterial biofilms. The samples were classified as: non-biofilm producer ($OD_{biofilm}$ < ODc-), weak biofilm producer (ODc- < $OD_{biofilm}$ ≤ 2 x ODc-), moderate biofilm producer (2 x ODc- < $OD_{biofilm}$ ≤ 4 x ODc-) and strong biofilm producer (4 x ODc- < $OD_{biofilm}$).

Biofilm formation evaluation of *Pseudomonas fluorescens* strain PL5.4 on stainless steel surfaces with residual milk

The biofilm formation capacity of the *P. fluorescens* PL5.4 strain on AISI 304 stainless steel test specimens (1 cm x 1 cm, negligible thickness) was evaluated according to the methodology described by Nörnberg et

al. (2011) and Bayoumi et al. (2012) with some modifications. The test was conducted in Erlenmeyer flasks containing TSB broth. In order to simulate the presence of milk on a stainless steel surface, the TSB broth was added with skimmed UHT cow's milk, before the biofilm formation assay.

To sanitize the stainless steel specimens, they were immersed in acetone for 30 minutes to remove any grease or fingerprints, then rinsed with distilled sterile water (dH_2O) and sterilized by autoclaving. For the experiment, bacterial colonies were grown in TSA for 24 h at 30 °C. They were transferred to TSB culture medium (pre-inoculum) and incubated for 24 h at 30 °C. A 2 mL aliquot at 10^8 CFU/mL (adjusted by a spectrophotometer to an optical density of 600 nm) was transferred to the Erlenmeyer flasks containing 18 mL TSB plus 0.25% glucose as well as to the Erlenmeyer flasks containing 16 mL TSB plus 0.25% glucose and 2 mL skimmed UHT milk. Three stainless steel specimens were added to each flask. They were subsequently incubated without shaking at 7 °C for 24, 48 and 72 h and every 24 hours a AISI 304 stainless steel specimen from each Erlenmeyer flask was aseptically removed using sterile forceps and washed three times in sterile dH_2O to remove poorly adherent cells. The specimens containing biofilm cells were immersed in 10 mL of 0.85% saline solution and subjected to the Unique Model USC700 ultrasound bath at a frequency of 40 kHz, and treated for a period of 10 minutes in order to release cells from the biofilm. Decimal dilutions were made from each solution containing the specimen. They were then plated and incubated at 30 °C for 24 hours. Counts were performed to determine the number of biofilm cells and planktonic cells, which were expressed in log CFU/cm^2 and log CFU/cm^3. All counts were performed in duplicate and each assay was repeated twice.

Evaluation of genomic sequence of *Pseudomonas fluorescens* PL5.4 isolated from raw buffalo milk in refrigeration tanks

Pseudomonas sp. belong to the family *Pseudomonadaceae* of the subclass *Gammaproteobacteria*. This isolate was first identified as *Pseudomonas fluorescens* by 16S rRNA and deposited under code MH046417 in the Standard Nucleotide BLAST (available at http://www.ncbi.nlm.nih.gov). Given the diversity of the complex, for better classification a complete bacterial genome sequencing was performed using the Nextera XT kit and the reagent cartridge MiSeq Reagent Kit v3 (150 cycles - 2 x 75 bp) in MiSeq equipment.

Statistical analysis

The counting values were converted to decimal logarithms (log CFU/cm^2; log CFU/cm^3) to correspond to a normal distribution. The results were submitted to an analysis of variance (ANOVA), using generalized linear models of the SPSS program, version 18, followed by the Bonferroni test to evaluate the significant differences, where the value $p<0.05$ was accepted as indicating significant differences.

RESULTS

Evaluation of bacterial motility using the twitching assay

In the evaluation of the *Pseudomonas fluorescens* PL5.4 and *Pseudomonas fluorescens* PL7.1 for twitching type motility, no area of motility was observed. The isolates were considered negative for this test. The area presented by the positive control ranged from 7 to 8 mm.

Evaluation of bacterial motility using the swarming assay

The *P. fluorescens* PL5.4 strain showed a difference of 80% in radial growth in 0.5% LB

agar when compared to the negative control in TSA and presenting a mean area of 21 mm in LB agar 0.5% and 13 mm in TSA. It was considered a positive test score. The *P. fluorescens* PL7.1 strain presented no difference in either of the tested media, which was considered a negative test result. The positive control presented a difference of 100% in the radial growth observed in the tested media.

Violet crystal assay for evaluation of biofilm formation with the presence of residual milk

In the presence of residual milk, it was observed that *Pseudomonas fluorescens* PL5.4 and *Pseudomonas fluorescens* PL7.1 showed similar behavior when submitted to different conditions. Both strains presented themselves as strong biofilm producers at 7°C for a period of 72 hours, moderate biofilm producers at 23°C for a 24 hours and non-producers at 37°C for a period of 24 hours. *S. epidermidis* ATCC 35984 positive control presented as strong biofilm producer at temperatures of 23 °C and 37°C and a non-producer at 7°C.

Biofilm formation evaluation of *Pseudomonas fluorescens* strain PL5.4 on stainless steel surfaces with residual milk

For *P. fluorescens* PL5.4 tested under different conditions, it was not possible to observe a significant difference ($p > 0.05$) in the number of cells that adhered to stainless steel in TSB broth medium or in TSB broth medium with residual milk. Also, no significant difference ($p>0.05$) was observed within the time ranges 24, 48 and 72 hours respectively. When temperature variations in the TSB broth were evaluated, the mean number of cells which adhered at 7 °C in 24 hours was 7,3 log CFU/cm^2. At 48 hours, the average number of adhered cells was 7,5 log CFU/cm$^{2.}$ At 72 hours, it was 7,4 log CFU/cm^2. When time variations in the TSB broth with residual milk were evaluated, the average number of adhered cells at 7 °C in 24 hours was 7,5 log CFU/cm^2. At 48 hours, the mean number of adhered cells was 6,8 log CFU/cm^2. At 72 hours, it was 7,2 log CFU/cm^2. Both results are shown in figure 1. The planktonic cell count at the initial inoculation (time 0), at a temperature of 7 °C, averaged a count of 8,5 log CFU/cm^3 for the TSB broth without added milk and 8,7 log CFU/cm^3 for the TSB broth with added residual milk. When time variations for the TSB broth without added milk were evaluated, at a temperature of 7 °C in 24 hours, the plankton cell count was 8,5 log CFU/cm^3. In 48 hours, the mean number of planktonic cells was 9,0 log CFU/cm^3. At 72 hours it was 9,9 log CFU/cm^3. When time variations for TSB broth with added residual milk were evaluated, in 24 hours, the planktonic cell count was 8,5 log CFU/cm^3. In 48 hours, the mean number of planktonic cells was 8,9 log CFU/cm^3. In 72 hours, it was 9,5 log CFU/cm^3. Both results are shown in figure 2.

Analysis of genomic sequence of *Pseudomonas fluorescens* PL 5.4

In this work it was possible to observe that *Pseudomonas fluorescens* PL 5.4 showed greater capacity in the formation of biofilms under the experimental conditions performed. Quality analysis was performed using FastQC Software Version 0.11.5. After filtering the sequences in Trim Galore! Version 0.4.4, the genome was assembled with Spades Version 3.10.1. The size of the assembled genome is 6,005,716 bp, with a content of 59.8% G + C, 209 scaffolds, 5,392 CDS, N50 size 35,560 bp and L50 51.

The *Pseudomonas fluorescens* PL5.4 isolate was identified on the basis of a 99% sequence identity with *Pseudomonas fluorescens* A506, a strain used as a biocontrol in agriculture (Saleem et al. 2018). The sequence was annotated with RAST (http://rast.nmpdr.org). The genome draft

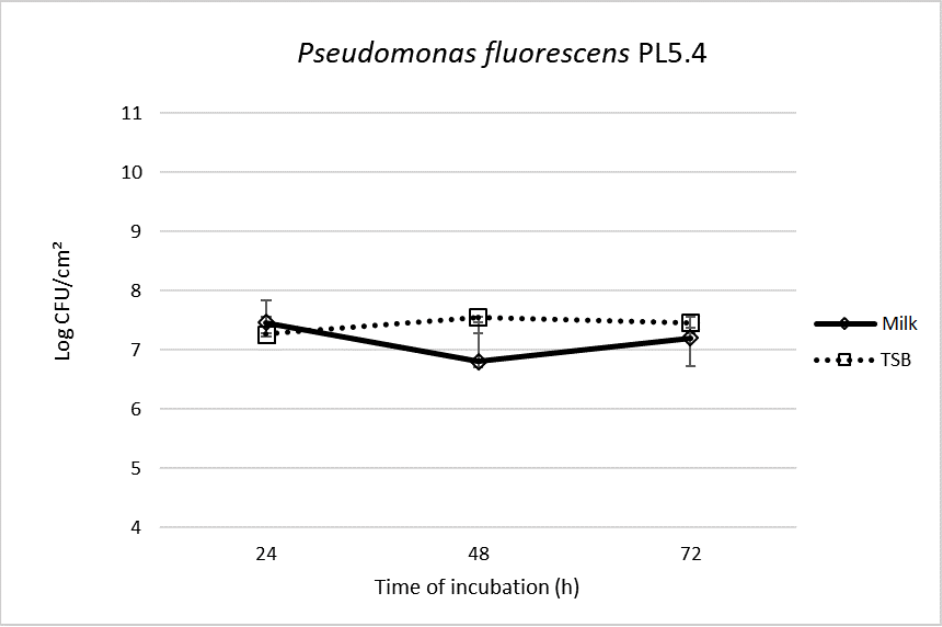

Figure 1. Adhesion of the *P. fluorescens* PL5.4 strain on a stainless steel surface in TSB broths, with and without added residual milk in 24, 48 and 72 hours at 7 °C.

was deposited in the NCBI under access number PPVD00000000.

DISCUSSION

Pseudomonas fluorescens PL5.4 and PL 7.1 were isolated from refrigeration tanks containing raw buffalo milk. *Pseudomonas* sp. is a gram-negative, mobile bacteria with flagellum that contains type IV pili (Yang et al. 2017). This pili presents two kinds of motility: the first is referred to as twitching motility (spasmodic motility), and is associated with the repetitive movements of extension and retraction of type IV pili structures. This can be observed on solid surfaces or on moderate viscosities (1% of agar). The second form of motility is swarming motility, which has a dendritic pattern of movement and is observed in the presence of specific sources of nitrogen and carbon, as well as on less viscous surfaces (0.4-0.7 % of agar) (Otton et al. 2017).

Twitching motility is only mediated by type IV pili, whereas swarming motility involves type IV pili as well as the secretion a class of surfactants known as rhamnolipids (Otton et al. 2017). Rhamnolipids produced during swarming act as surface humidifiers and contribute to adhesion and virulence (Lakshmanan et al. 2018). Swarming and twitching motility types have been associated with the development of *P. aeruginosa* biofilm, which in turn has become a model organism for research on type IV pili and biofilm (Otton et al. 2017). Twitching motility is a movement typical of type IV pili by which bacteria can adhere to and form biofilms on a surface and is controlled by the quorum sensing in *P. aeruginosa* (Ribbe et al. 2017). Type IV pili are an essential step towards irreversible adhesion, it may affect the morphology and structure of the biofilm (Sun et al. 2017). However, contrary to what is found in *P. aeruginosa* species, *P. fluorescens* strains PL5.4 and PL7.1 did not present twitching motility. As type IV pili are unique cell structures involved in this type of motility, it is possible to assume that this structure may be absent or unable to function properly. The reasons for this observation may be the absence of genes responsible for forming the pili structures or genes which may be present but are unable

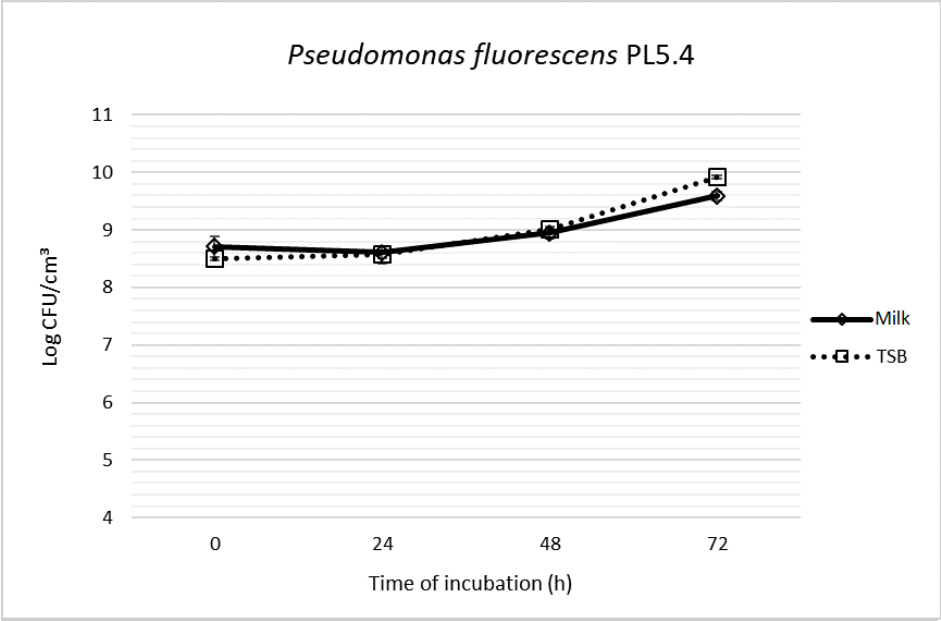

Figure 2. Planktonic cell count of the *P. fluorescens* PL5.4 strain in TSB culture medium without added residual milk and in TSB culture medium with residual milk in 24, 48 and 72 hours at 7 °C.

to express as desired, or even genes that are present, but in mutated form (Otton et al. 2017).

For the swarming motility assay, only the *P. fluorescens* PL5.4 strain obtained a positive score. This type of motility is a complex phenomenon involving several factors besides rhamnolipids, flagella and collaboration with type IV pili (Lakshmanan et al. 2018). This allows us to conclude that the strain which has no twitching motility, may still present swarming movement, once this motility is related to the presence of flagella and other factors (Mastropaolo et al. 2012). Studies have shown that mutants deficient in swarming motility were defective in the production of lipopolysaccharides, while some were deficient in the production of c-di-GMP signaling systems or factors that regulate cell differentiation (Yang et al. 2017, Lakshmanan et al. 2018). The absence of swarming motility in the *P. fluorescens* PL7.1 strain may have been mediated by the abovementioned factors, including a reduction in surface moisture, a decreased production of exopolysaccharides or a defect in binding protein synthesis.

In order to have a better understanding of the impact of motility on biofilm formation, it is of the utmost importance to perform tests for the genes related to type IV pili, in strains that have negative results for them but are considered to be strong biofilm producers (Otton et al. 2017). If the presence of these genes is confirmed, then there is the possibility that the conditions under which the motility tests were performed may have influenced gene expression (Oliveira et al. 2016).

Bacterial contamination can impair the quality, functionality and safety of milk and its derivatives (Rossi et al. 2016). It is known that the main source of contamination of dairy products is often associated with the formation of biofilms on the surfaces of milk processing equipment (Ben-Ishay et al. 2017). The present study showed that both *P. fluorescens* PL5.4 and PL7.1 strains were able to form biofilm on microplates in the presence of residual milk. Both strains were moderate biofilm producers at room temperature and strong biofilm producers at a temperature of 7 °C, which is the temperature at which milk is stored. Both were non-biofilm producers at

37 °C. This may have occurred because their optimal growth temperature was around 28 °C (De Jonghe et al. 2011). Rossi et al. (2016) reported that the ability to produce biofilm from its isolates may be associated with low temperature. As previously described, biofilm formation is enhanced by cell motility and by certain environmental conditions (Ribbe et al. 2017). As such, another possibility is that the ability of the studied strains to produce type IV pili and produce biofilms may depend on the temperature used, since for the motility test only a temperature of 30 °C was tested in this study.

The *P. fluorescens* PL5.4 strain, which was chosen for presenting the best results in the motility test, was able to produce biofilms on a stainless steel structure and maintain this structure for up to 72 hours. Similar situations can be found in the food industry. The existence of these biofilms on stainless steel surfaces containing milk has major implications for the industry, because of their ability to adhere to the surfaces of milking equipment and pipes and production system filters, and in this way becoming a constant source of contamination (Puga et al. 2016, Ben-Ishay et al. 2017). Not even the use of lower temperatures (4-10 °C) prevents biofilm formation, probably because these bacteria have already adapted to these lower temperatures under storage conditions (Puga et al. 2016, Rossi et al. 2016*)*.

The dairy sector is one of the main sectors impacted by food loss, since nearly 20% of conventionally pasteurized fluid milk is discarded (Cleto et al. 2012). Bacterial enzymes may have a damaging effect on dairy products during prolonged storage and can reduce or alter the physical and chemical properties of the product, thus causing defects in the functionality and sensory properties (Nörnberg et al. 2011). Some bacteria are able to produce thermostable enzymes, which persist after the milk pasteurization process (De Jonghe et al. 2011). Biofilm formation may have undesirable implications because the releasing of biofilm from a surface during processing may contribute to the contamination of the finished product. Such contamination may cause cheese deterioration and reduce the shelf life of the derived products (Rossi et al. 2016, Ben-Ishay et al. 2017).

More information on biofilms can be helpful in developing strategies to eradicate them successfully. More efficient practices of hygiene should be adopted to prevent and limit the development of psychrotrophic bacteria, in order to reduce the losses of raw material due to the deterioration of dairy products.

Studies addressing bacterial motility and biofilm production by *Pseudomonas fluorescens* are extremely important in order to understand these mechanisms and thus be able to find ways of intervening in the control of bacterial growth and in the formation of these structures. The present study reinforces the importance of the prevention and eradication of biofilms that pose a threat to the quality of dairy products.

With the identification of *Pseudomonas fluorescens* PL 5.4, as a potential biofilm-forming agent in the experimental conditions studied, and for presenting swarming motility, this culture was selected for the study of its genome. Its genome was sequenced and it was found to share 99% sequence identity with the *Pseudomonas fluorescens* A506 genome sequence. *Pseudomonas fluorescens* can often be isolated from refrigerated raw milk and play an important role in the deterioration of food because they are able to produce thermoresistant enzymes and form biofilms (Rossi et al. 2016). In a study by Nucera et al. (2016),

they confirmed the presence of *P. fluorescens* (45% of the isolates) as the predominant species in the dairy environment. *Pseudomonas* is one of the most complex and diverse genera, which is reflected in the more than 100 species which have been described (Garrido-Sanz et al. 2016, Tran et al. 2017). New taxonomy studies using genotypic tests demonstrate that many isolates identified as *Pseudomonas fluorescens* are being reclassified as new species within a *Pseudomonas fluorescens* "complex". There are currently 52 species within this complex, sharing many phenotypic characteristics (Scales et al. 2014, Garrido-Sanz et al. 2017). They were divided into subgroups that differ in multilocus sequence analyses (MLSA) and phylogenetic analyses. The *Pseudomonas fluorescens* complex contains 8 phylogroups: *Pseudomonas mandelii, Pseudomonas jessenii, Pseudomonas koreensis, Pseudomonas corrugata, Pseudomonas fluorescens, Pseudomonas gessardii, Pseudomonas chlororaphis* and *Pseudomonas protegens* (Garrido-Sanz et al. 2017). The difficulties surrounding this complex are frequent descriptions of new species and the inclusion of new strains (Garrido-Sanz et al. 2016).

Still, from the information generated, we will seek to study its genome in more detail looking for genes related to the properties identified in this work. Knowing environmental microorganisms is an important point of investigation, considering their transmission through an important food source such as milk.

Considering the study carried out, it is important to highlight the importance of the Good Practices programs in the Dairy Industry and the Analysis of Hazards and Critical Control Points. If properly applied, they can prevent several problems in technological processes and in the processing environment. Special attention should be given to raw milk storage tanks in conjunction with Good Agricultural Practices (Chon et al. 2021).

CONCLUSIONS

The study of factors related to the production of microbial biofilms seeks to elucidate the mechanisms inherent in the environment and the microorganisms involved. *Pseudomonas fluorescens* is a deteriorating microorganism and is normally present in whole milk and its proliferation should be avoided based on good agricultural practices. It is important to highlight that *Pseudomonas fluorescens* is a commensal bacterium present at low level in the human digestive tract that has also been reported in many clinical samples (blood, urinary tract, skin, lung, etc.) and sometimes associated with acute opportunistic infections. The *Pseudomonas* species related to mastitis is *Pseudomonas aeruginosa*.

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