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Detection of microbiota in cow's milk with a focus on the genus *Pseudomonas*

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Abstract: *In this study, raw cow's milk samples were analyzed to detect Pseudomonas bacteria, recognized as indicators of milk hygiene and contributors to mammary gland inflammation. The research was conducted on four production farms (PF1, PF2, DH3, and DH4) in the Slovak regions of Abov, Spiš, and Zemplín, under practical conditions. Milk samples were collected seasonally during autumn 2022, winter 2022, spring 2023, and summer 2023. Out of 320 raw milk samples subjected to comprehensive quality and microbiological analysis, 73 isolates of Pseudomonas spp. were identified using PCR techniques. The breakdown of species was as follows: P. putida (34.25%), P. fragi (13.70%), P. lundensis (9.59%), and other Pseudomonas spp. (42.47%). These results underscore the importance of Pseudomonas spp. as a key factor in assessing milk quality and their implications for udder health in dairy cattle.*

I. INTRODUCTION

Representatives of the genus *Pseudomonas* belong to Gram-negative, aerobic rods with a size of 0.5 - 1.0 x 1.5 - 5.0 μm. They do not form spores, they move with the help of one or more polar flagella. Most representatives are oxidase and catalase positive with the exception of *Pseudomonas* oryzihabitans and *Pseudomonas luteola*. They are important from a food point of view, as many representatives survive and grow at an ambient temperature of around 4 °C. Their negative impact on the quality of food consists in the production of thermostable lipolytic and proteolytic enzymes, which lead to the degradation of fats and proteins with subsequent food spoilage [1].

The main agents causing inflammation of the mammary gland include the opportunistic pathogen *Pseudomonas aeruginosa*, which, in addition to mastitis (clinical, subclinical, chronic), is capable of causing infection in any tissue or organ [2]. *Pseudomonas fluorescens* is a G-negative, rod-shaped bacteria that inhabits soil, plants, and water surfaces. *Pseudomonas fluorescens* is the most common type of *Pseudomonas* spp. that causes milk spoilage. Comprising a group of common non-pathogenic saprophytes, it secretes a soluble greenish fluorescent pigment called fluorescein, especially under conditions of low iron availability [3]. *Pseudomonas putida* strains are often multidrug resistant, and clinical isolates of *Pseudomonas putida* are considered an environmental reservoir of resistance determinants [4]. In the dairy industry, it negatively affects the organoleptic properties of milk. It can cause off-odors or off-tastes in pasteurized milk and other dairy products [5].

In samples of raw cow's milk, we focused on determining the presence of bacteria of the genus *Pseudomonas*, which are an indicator of the hygienic quality of milk and can contribute to the development of inflammation of the mammary gland.

II. Materials and Methods

From four production farms (PF1 – PF4), n = 320 samples of raw cow's milk were taken during the period (autumn 2020 to summer 2021). We obtained a total of 151 isolates by culturing microbiological examination.

The PCR method according to Yamamoto and Harayama [6] was used to determine the genus Pseudomonas as well as individual species; Spilker et al. [7], He et al., [8], Bedeltavana et al., [9], Lurthu and Reetha et al., [10].

As a positive control, the following reference strains were used for PCR: *Pseudomonas aeruginosa* CCM 1960T, *Pseudomonas fluorescens* CCM 4795, *Pseudomonas fragi* CCM 1974T, *Pseudomonas putida* CCM 7156T, *Pseudomonas lundensis* CCM 3503T (Czech collection of microorganisms, Brno, Czech Republic).

III. Results

The presence of the 16S rDNA gene, which is typical for the genus *Pseudomonas* spp. 73 isolates of a selected group of microorganisms *Pseudomonas* spp. were confirmed using the PCR method.

Individual samples of milk taken from production farm 1 were negative for determining the presence of the selected group of microorganisms *Pseudomonas* spp. during the entire experimental period (0%). In the milk samples taken from PH2/ PF2, we detected the presence (23.29%; 17 isolates) of *Pseudomonas* spp. We detected the largest collection of *Pseudomonas* spp in PH4/ PF4 (47.95%; 35 isolates). The representation of *Pseudomonas* spp. isolates in individual production farms is shown in Figure 1.

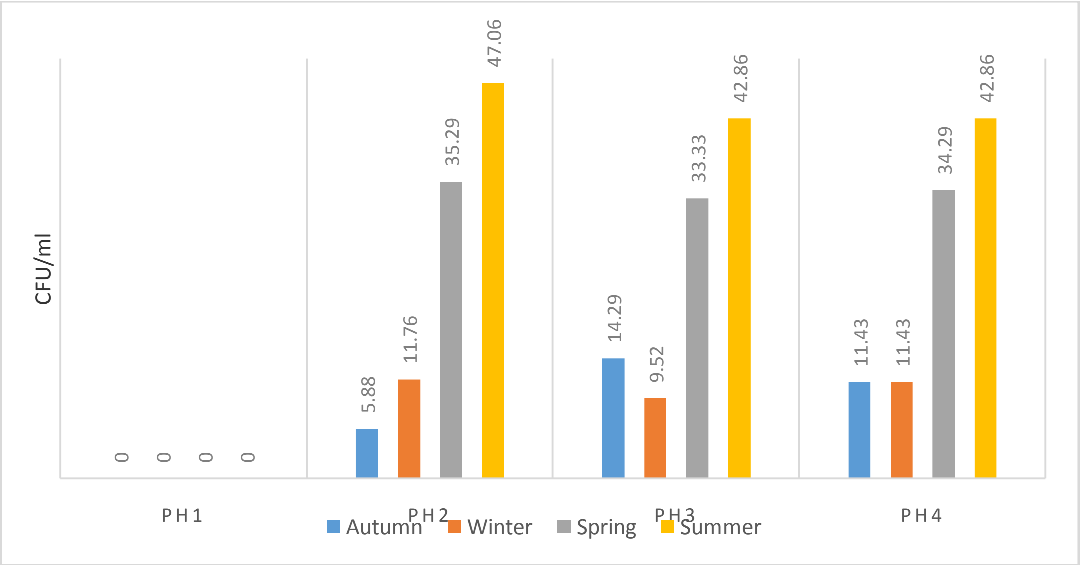

Figure 1. Seasonal representation of *Pseudomonas* spp. (PH/ PF)

Species identification of isolated and confirmed strains of *Pseudomonas* spp. we also performed using the PCR method. Based on species identification, we determined the following three species of selected microorganisms of the Pseudomonas group.

From a total of 73 confirmed strains, the following species were identified: *Pseudomonas putida* (34.25%; 25 isolates); *Pseudomonas fragi* (13.70%; 10 isolates); *Pseudomonas lundenses* (9.59%; 7 isolates). *Pseudomonas* spp. remained unconfirmed (42.47%; 31 isolates). (Figure 2). The presence of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* species we did not detect raw cow's milk in any of the collected samples (0%).

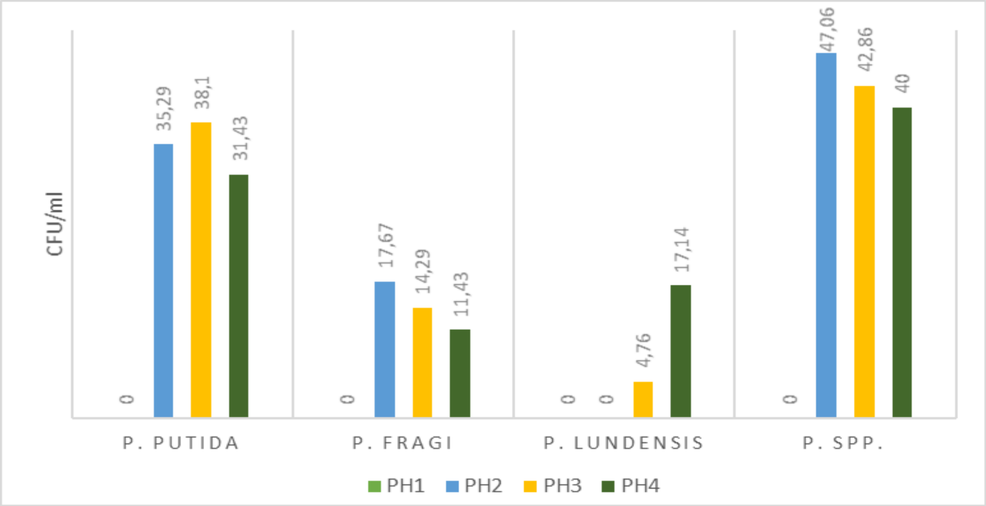

Figure 2. Representation of individual species of the genus *Pseudomonas* in all 4 production farms PH/ PF 1 – PH/ PF4

IV. Discussion

The class of Gammaproteobacteria, especially the genus *Pseudomonas*, constitutes a significant part of the microbiota of raw milk. Both fluorescent and non-fluorescent pseudomonads are considered a major cause of spoilage of milk and other dairy products. In addition, the genus *Pseudomonas*, other gammaproteobacteria such as *Acinetobacter*, *Aeromonas*, *Stenotrophomonas*, *Hafnia*, *Rahnella* and *Serratia* were frequently detected in raw milk in studies by other authors [11]. Among the most frequently isolated species are Pseudomonas spp. in milk and dairy products include: *P. fluorescens*, *P. fragi* and *P. lundensis* [12].

This information on the species prevalence of representatives of the genus *Pseudomonas* also adjusts the results of our work, where we isolated a total of (n = 73) isolates of *Pseudomonas* spp., which were represented by species as follows: *Pseudomonas putida* (34.25%) *Pseudomonas fragi* (13.70%); *Pseudomonas lundenses* (9.59%).

Based on PCR Sequencing [13], in their work determined that 42.7% (n = 61) isolates from milk samples consisted of *P. fluorescens*, the dominant *Pseudomonas species*. Marchand et al. [14] indicated that the most frequently occurring species isolated from milk were *P. fragi* and *Pseudomonas lundensis*. Caldera et al. [15] detected the following *Pseudomonas* species in raw milk: *P. fragi*, *P. gessardii*, *P. proteolytica*, *P. brenneri*, *P. rhodesiae* and *P. peli*.

Due to its composition, milk mainly creates a nutrient medium that is easily subject to microbial contamination. The number of psychrotrophic microorganisms represents less than 10% of the total microbiota in milk obtained under hygienic conditions. Bacteria of the genus *Pseudomonas* spp. with their presence, they participate in reducing the hygienic properties of milk.

V. Conclusion

Although *Pseudomonas* spp. are among the ubiquitous bacteria that are often isolated from raw milk, our findings indicate that the species representation of *Pseudomonas* spp. present in milk vary considerably from study to study. These differences may result from regional and environmental differences. It is important to focus on each production farm individually because of the different management practices implemented at the farm level.

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