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NALAZ PSEUDOMONAS SPP. U SIROVOM MLEKU
OCCURRENCE OF PSEUDOMONAS SPP. IN RAW MILK

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Rod *Pseudomonas* obuhvta gram-negativne štapiće, koji nisu zahtevni u pogledu hranjivih sastojaka neophodnih za rast i naseljavaju brojne ekološke niše. Često su izolovani iz hrane, zemlje, štalskog đubriva i sa kože životinja. Svrstavaju se u oportune patogene mikroorganizme, jer mogu da izazovu akutne, ili hronične infekcije različitih domaćina-sisara i njihovih organa zbog sposobnosti stvaranja širokog spektra faktora virulencije. *Pseudomonas* vrste dobro rastu na bakteriološkim podlogama i često stvaraju pigment, i to *P. aeruginosa* termostabilni plavo-zeleni pigment (*pyocyanin*) i *P. fluorescens* zelenkasto-žuti pigment fluorescin. Značaj sa aspekta higijene mleka se ogleda u tome što, iako se ovaj psihrotrofni mikroorganizam uništava procesom pasterizacije, može da stvara termostabilne metaboličke produkte i enzime, koji izazvaju kvar proizvoda od mleka. Najčešće izolovan je *P. aeruginosa*, ali i *P. fluorescens*, koji dovodi do kvara maslaca i UHT mleka.

CILJ ovog rada je bio da se ukaže na značaj *Pseudomonas* vrsta kao konatminenta mleka i mogućeg uzročnika kvara.

Materijal je predstavljalo 7 uzoraka zbirnog mleka sa brojem somatskih ćelija manjim od 400.000/ml. Nalaz i identifikacija je rađena tokom rutinskog ispitivanja sirovog mleka metodom ISO 6579-1:2017. Kolonije na XLD agaru koje su bile laktoza negativne sa ili bez H₂S, a sa, ili bez plavičaste boje oko kolonija i karakterističnim mirisom dalje su biohemijski ispitivane. Krvni agar je korišćen za ispitivanje hemolitične aktivnosti, a podloga sa kazeinom i tributirin agar i za ispitivanje proteolitičke i lipolitičke aktivnosti.

Rezultati: Od 7 uzoraka mleka *Pseudomonas* spp. je dokazan u 6 (85,71%) uzoraka sirovog mleka. Svih 28 izolati su oksidaza i katalaza pozitivni, stvarali su β hemolizu na krvnom agaru, proizvodili su pigment na Tripton soja agaru (TSA) i imali karakterističan miris. Svih 28 izolata je pokazalo proteolitičku i lipolitičku aktivnost. Najčešće izolovan je *Pseudomonas fluorescens*, a dva izolata *Pseudomonas aeruginosa* su stvarala zeleni pigment *pyocyanin*.

ZAKLJUČAK: Iako zakonska regulativa u Republici Srbiji ne predviđa ispitivanje uzoraka mleka na prisustvo *Pseudomonas* vrsta, moguć je nalaz ovog mikroorganizma u sirovom mleku, kao posledica kontaminacije.

KLJUČNE REČI: mleko, *Pseudomonas* spp., kvar

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The genus *Pseudomonas* includes gram-negative bacilli, not requiring nutrient compounds necessary for growth and inhabit numerous ecological niches. They are often isolated from food, soil, manure and animal skin. They are classified into opportunistic pathogenic microorganisms because they can cause acute, or chronic infections of various mammalian hosts and their organs due to the ability to form a wide spectrum of virulence factors. *Pseudomonas* species grow well on bacterial media and often produce pigment, such as *P. aeruginosa*-thermostable blue-green pigment (*pyocyanin*) and *P. fluorescens*-greenish-yellow pigment fluorescin. From the view point of milk hygiene the importance of this psychrotrophic microorganism is, even it is destroyed by the pasteurization process, ability to synthesize thermostable metabolic products and enzymes that cause spoilage of milk products. The most commonly isolated is *P. aeruginosa*, but also *P. fluorescens*, which leads to spoilage of butter and UHT milk. The aim of this research was to point out the significance of *Pseudomonas* species as the milk contaminant and the possible cause of spoilage.

The material represented seven bulk tank milk samples in which the somatic cell counts were less than 400,000/ml. The isolation and identification was carried out during the routine testing of raw milk by the method ISO 6579-1: 2017. Colonies on XLD agar that were lactose negative with or without H₂S, and with or without blue pigmentation around the colonies and characteristic smell were biochemically examined. The blood agar was used for the examination of haemolytic activity, and the casein agar and tributirin agar for the determination of proteolytic and lipolytic activity, respectively.

Results: Out of 7 milk samples *Pseudomonas* spp. was detected in 6 (85.71%) samples of raw milk. All 28 isolates were oxidase and catalase positive, they produced β hemolysis on blood agar, they produced a pigment on Tryptone soy agar (TSA) and had a distinctive smell. All 28 isolates showed proteolytic and lipolytic activity. The most commonly isolated is *Pseudomonas fluorescens*, and two *Pseudomonas aeruginosa* isolates produced green pigment-*pyocyanin*.

CONCLUSION: Although according to the legislation in the Republic of Serbia does not provide the examination of milk samples for the presence of *Pseudomonas* species, it is possible to find this microorganism in raw milk as a result of contamination.

KEY WORDS: milk, *Pseudomonas* spp., spoilage