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Research Article

Occurrence of Pseudomonas Aeruginosa in Homemade Fresh Butter in Some Rural Areas in Kosovo

Healthcare

Keywords: Pseudomonas aeruginosa, butter, fresh, Kosovo.

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Abstract

This study is carried out from 2014 to 2015 in some rural areas in Kosovo. From the analytical control samples of 87 fresh butter sampled collected from rural areas of Ferizaj and Gjilan in Kosovo are isolated 8% Pseudomonas spp strains. Further differentiation of isolated strains confirmed Pseudomonas aeruginosa in 4, 2% of collected samples. The identification of isolates was succeeded using planting techniques and peptone broth as well as selective media Pseudomonas CFC agar. All isolated strains produced protease and lipase by only 3 strains. Butter is stored in cold temperature at 4o C. In such environment Pseudomonas aeruginosa cells are under inhibitory effects of low temperatures but they can survive. Inhibitory effects in milk cultures (cream and yogurt) have been observed in some cases of contamination of milk products with Pseudomonas aeruginosa.

Introduction

Pseudomonas aeruginosa is a Gram-negative bacterium in the form of rod belonging to the family *Pseudomonadaceae***.** *Pseudomonas aeruginosa* is a species of this group, which includes 12 members. Like other members of the genus, *Pseudomonas aeruginosa* is a free bacterium, commonly found in soil and water [2, 3, 8]. But this bacterium is regularly on surfaces occasionally in plant and in animal body surfaces. However, *Pseudomonas aeruginosa* has become increasingly recognized as an emerging opportunistic pathogen of clinical importance. Various studies have analyzed epidemiological occurrence as a nosocomial pathogen that show its increasing resistance to antibiotics in clinical isolates. This group of organisms has the advantage to evolve in organisms and to weak immune system but it can withstand well an individual's immune system consolidating the infection associated with the production of harmful toxins. *Pseudomonas spp* is typically found in outdoor surface or substrate, or in the form of colonies or unicellular organism [5, 10, 16, and 17]. Pseudomonas is problematic bacterium in environmental and drinking water. In the laboratory, *Pseudomonas aeruginosa* grows in an environment that consists on use of acetate as a carbon source and ammonium sulfate as nitrogen source. *P. aeruginosa* possesses the metabolic versatility for which these bacteria are known. Organic growth factors are necessary, and it can use more than seventy-five organic compounds for growth [1, 4, and 18]. The optimum temperature for its growth is 37^{o}C, and the bacterium is able to grow at high temperatures up to 42^{o}C being tolerant of a wide variety of physical conditions, including temperature. This bacterium is resistant to high salt concentrations and colors, weak antiseptics, and many commonly used antibiotics. *Pseudomonas aeruginosa* has a predisposition to be growth in moist environments, which is probably an adaptation to his natural life in soil and water. These natural habitats of this bacterium undoubtedly contribute to its ecological presence as a food opportunist pathogen [11, 16, and 19]. Isolates of *P. aeruginosa* produce three types of colonies. Natural isolates from soil or water typically produce smaller colonies. Clinical samples, in general, give two types of colonies. One type has a fried egg like that is great, soft, flat edges and overlooking set. Another type, isolated from respiratory tract secretions and urine, has a mucoid and production attributed to agglutinates [7, 12]. Mucoid colonies are supposed to play a role in colonization and virulence of this pathogen. *Pseudomonas aeruginosa* infects cows causing a more serious infection in the mammary glands. These bacteria can attack cows' immune system causing wounds or other infection. Poor nutrition also causes cow to be susceptible to infections. Non-clinical infections are more likely to develop if herd is exposed to a small number of bacteria over a period of time. The most common environments for its growth is water to be used for washing.

The most frequent source of contamination is water from wells used for treating breast. Some of the signs of mastitis caused by *Pseudomonas aeruginosa* include a sudden outbreak of this infection in some cows in a few days, but no clinical difference. This pathogen is also found in dairy products like creams, butter and cheese. According to some research data given by some authors, there is an evidence of this pathogen in fresh butter with the incidence ranges from 3-27% in countries like Turkey and Egypt [15, 16].

Recently, in some conducted studies in developed countries epidemiological data reported incidence of *Pseudomonas aeruginosa* ranging from 2-12% in a raw milk and butter samples [8, 15].

Material and Methods

From 2014 to 2015, a total of 87 butter samples produced in home conditions are collected in Aseptic conditions from various houses of farmers in rural areas in the regions of Ferizaj and Gjilan in Kosovo. All samples were taken in sterile glass containers and are transported under refrigeration conditions to the laboratory. Samples were analyzed for the presence of *Peseudomonas spp*. The analytical processes of butter samples are performed initially by diluting 1 to 10 with 1% butter with sterile peptone water and were homogenized using a laboratory mixer. A volume of sample dilution is inoculated in Pseudomonas CFC selective agar. Sample volumes are widespread by throwing on the surface 0.5 or 1 ml volume of the homogenized samples on the selective plate spreading over the surface with a sterile spatula. The inoculated plates are incubated at 25 ° C and are examined after 24 and 48 hours at room temperature, using white light and ultraviolet light. The Pseudomonas CFC agar is a good media for growth of *Pseudomonas spp* but usually is limited to a few members of the family *Enterobacteriaceae* can be present. The presence of blue colonies, in green, yellow, or brown pigmentation as well as the fluorescence can be taken as evidence for the presence of *Pseudomonas spp* but further tests should be carried out to confirm and identify the organism. Pseudomonas modified media as CFC agar reached differentiation of *Pseudomonas spp* from *Enterobacteriaceae* species. From the development of broth cultures incubated in reduced atmosphere was added 1% arginine and red phenol in 0.002% of selective media. *Pseudomonas* organisms produce ammonia from arginine and are distinguished by the color of pink colonies [9]. For isolated cultures are used preliminary tests and some other additional tests for further identification. For this purpose are used agar nutrient or blood agar plates. The inoculated plates are placed for 18 to 24 hours at 37^{o} C or at room temperature (24 to 28^{o} C) depending on optimal growth requirements of the organism. For confirmation of cytochrome oxidase in culture broth was added 0.2 ml of solution 1-naphtol and 0.3 ml of p- P-Aminodimethylaniline oxalate in each tube. The tubes were shaken to ensure complete mixing and oxygenation of the culture. The appearance of a blue color indicates the presence of cytochrome oxidase in bacterial cells [7, 9]. To detect the presence of this enzyme of microorganisms cultivated on agar, equal amounts of reagent was mixed with material from isolated colonies. Colonies were tested for survival in concentration of 7%.

This organism does not grow in high concentrations of NaCl. Pseudomonas strains are identified as Pseudomonas aeruginosa testing of commercial colonies on agar cream milk (skim milk agar) in temperature of 35°C for 24 hours. In the prepared plates was inoculated with loop 4 cm length of material from the suspected colonies. A translucent zone in the center with a green suburb in yellow color is caused by hydrolization of casein caused by *Pseudomonas aeruginosa.*

Results and Discussion

Table 1. Data analysis were conducted for the control of fresh butter samples for the presence of *Pseudomonas aeruginosa*

Regions	Fresh butter samples	Found positive with *Pseudomonas spp*	Found positive with *Pseudomonas aeruginosa*
Ferizaj	41	9, 5% (4/41)	4, 3% (2/41)
Gjilan	46	6.5% (3/46)	4, 1 % (2/46)
Total	87	8% (7/87)	4.2% (4/87)

Fig. 1. Image of casein test produced by *Pseudomonas aeruginosa* strains in cream milk agar.

In rural areas in Kosovo women typically use their traditional knowledge for homemade fresh butter. This product is used as fresh butter, but also it is for food preparation or cooking as well as other gastronomic purposes. Fresh butter used for cooking is kept 2-3 days at room temperature. The excess amount of sodium chloride added to the outer surface prolonged the shell life in domestic situations. The butter is produced manually and is stored in refrigerators until is being sold in the market or it can be held up to a week. Butter is not stored for long time because this product can be contaminated by spoilage bacteria and fungi (5, 8, and 10). The main source is butter cream, sweet or sour, raw or pasteurized. Yeasts and fungi are important organisms in breaking the butter and causes spots on the surface. Gram negative bacteria cause psychrotrophic and proteolytic changes in this product [7, 9]. Microbiological analysis of butter for certain pathogens as Escherichia coli and *Staphylococcus aureus* as well as for coliform bacteria are considered justified as limited tests. There also are many studies conducted in Egypt reporting data on the microbiological quality of the fresh butter for cooking [15, 16]. However, the data from recent studies about microbiological quality of butter are unclear or absent. Therefore, fresh butter for cooking is checked for the presence of *Pseudomonas aeruginosa*. The data obtained from the analysis carried out appeared that 8% of the samples were contaminated with *Pseudomonas spp* and identification of isolates resulted in 4.2% of samples contaminated with *Pseudomonas aeruginosa*. Different studies on fresh butter provide data that the presence of *Pseudomonas Arureginosa* ranges from 2-27% [14, 16]. In developing countries data on the incidence of *Pseudomonas aeruginosa* are reported in values from 6 to 12% lower than data in the above mentioned countries [10, 16, 18, and 19].

Conclusions

Results indicate that fresh butter in Kosovo is not produced in good hygienic conditions. Contamination of homemade butter with *Pseudomonas aeruginosa* poses a risk to public health. Therefore, there is a necessity for the development of hygienic production status for homemade butter in rural areas improving women's education and implementing the hygienic rules in the process of butter production. It is important for consumers to be known with storage methods of butter to be protected from contamination of various pathogens. Also,

butter should not be produced from raw cream; if it is produced from a raw cream then it should be used only for cooking after receiving appropriate heat treatment.

References

1. Anjaiah V, Koedam N, Nowark-Thompson B, Loper JE, Hofte M, Tambong JT, Cornelis P. Involvement of phenazines and anthranilate in the antagonism of *Pseudomonas aeruginosa* PNA1 and Tn5 derivatives toward *Fusarium sp* and *Pythium sp*. Molecular Plant-Microbe Interactions. 1998; 11: 847-854.
2. Palleroni, NJ. Genus I. *Pseudomonas* Migula. 1894; p. 141-199.
3. Stolp H, and Gadkari D. Nonpathogenic members of the genus Pseudomonas. 1981; p. 719-741.
4. Milton NS, Hilderbrand DC and Starr MP. Phytopathogenic members of the genus *Pseudomonas*. 1981; p. 701- 708.
5. Erskine RJ, Unflat JG, Eberhart RJ, Hutchinson LJ, Hicks CR, Spencer SB. *Pseudomonas* mastitis: Difficulties in detection and elimination from contaminated wash-water systems. J. Am. Vet. Med. Assoc. 1987; p. 191:811.
6. Frank JF. Milk and dairy products. *In* Food Microbiology, Fundamentals and Frontiers, ed. Doyle MP, Beuchat LR., Montville TJ, ASM Press, Washington. 1997; p. 101.
7. Jay JM. Taxonomy, role, and significance of microorganisms in food. *In* Modern Food Microbiology. Aspen Publishers, Gaithersburg MD. 2000; p. 13.
8. Kirk JH and Bartlett PC. Nonclinical *Pseudomonas aeruginosa* mastitis in a dairy herd. J. Am. Vet. Med. Assoc. 1984; p.184:671.
9. Malmo J, Robinson B, Morris RS. An outbreak of mastitis due to *Pseudomonas aeruginosa* in a dairy herd. Aust. Vet. J. 1972; p. 48:137.
10. Ombaka EA, Cozens RM, Brown MRW. Influence of nutrient limitation of growth on stability and production of virulence. Factors of mucoid and nonmucoid strains of *Pseudomonas aeruginosa*. Rev. Infec. Dis. 1983; 5:880.
11. Packer RA. Bovine mastitis caused by *Pseudomonas aeruginosa*. J. Am. Vet. Med. Assoc. 1977; p. 170: 1166.
12. Ray B. Spoilage of Specific food groups. *In* Fundamental Food Microbiology, CRC Press. Boca Raton FL. 1996; p. 220.
13. Vela GR. Microbiology of milk. *In* Applied Food Microbiology, Star Publishing, Belmont, CA, 1997; p. 325.
14. Khalafalla SM, El-Sadek GM, Teama ZY, Sultan NE. Microbiological studies on market butter in Egypt. Egyptian J Dairy Sci.1974; 2(2):119–126.
15. Sadek ZI, Abd El-Khalek A, Ibrahim B, GAM. Contamination and inactivation of *Pseudomonas aeruginosa* in some dairy products. Annals of Agricultural Science Cairo. 2006 ; 51 (1): pp.155-169.
16. Munsch-Alatossava P, Alatossava T. Phenotypic characterization of raw milk-associated psychrotrophic bacteria, Microbiological Research. 2006; 161, 4 (17): p. 334–346.
17. Manjiri V, Suryavanshi and Ghosh Jai S. Spoilage of White Unsalted Butter by Psychrophilic Lipolysis of Pseudomonas aeruginosa NCIM 2036. British Journal of Dairy Science. 2010; 1(1): 26-29, 2010.
18. Capodifoglio E. Centola AM, Joyce V, Bortoletto F, D'Abreu LF , Siqueira AC, Gonçalves A, Cristina A, Vaz N, Cesar J, Balieiro de C , Nett AS. Lipolytic and proteolytic activity of *Pseudomonas* sp isolated during milking and storage of refrigerated raw milk. Journal of Dairy Science. 2016: 99 (I 7): p.5214–5223.
19. Pereira S G, Mendez IP, Rosa AC and CardosOlga. Susceptibility to ultraviolet light C of *Pseudomonas aeruginosa* biofilms from hydropathic respiratory treatment equipments: impact in water quality control and public health. International Journal of Current Microbiology and Applied Sciences. 2015; 4(5): p. 1200-1208.