Spoilage potentials and antimicrobial resistance of *Pseudomonas* spp. isolated from cheeses

S. Arslan,¹ A. Eyi, and F. Özdemir

Abant İzzet Baysal University, Faculty of Arts and Sciences, Department of Biology, 14280, Gölköy/Bolu, Turkey

ABSTRACT

Pseudomonas spp. are aerobic, gram-negative bacteria that are recognized as major food spoilage microorganisms. A total of 32 (22.9%) *Pseudomonas* spp. from 140 homemade white cheese samples collected from the open-air public bazaar were isolated and characterized. The aim of the present study was to investigate the biochemical characteristics, the production of extracellular enzymes, slime and β -lactamase, and antimicrobial susceptibility of *Pseudomonas* spp. isolated from cheeses. The identified isolates including *Pseudomonas pseudoalcaligenes*, *Pseudomonas alcaligenes*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* biovar V, and *P. pseudoalcaligenes* ssp. *citrulli* were found to produce extracellular enzymes, respectively: protease and lecithinase production (100%), and lipase activity (85.7, 42.9, 100, and 100%, and nonlipolytic, respectively). The isolates did not produce slime and had no detectable β -lactamase activity. The antimicrobial susceptibility of the isolates was tested using the disk diffusion method. *Pseudomonas* spp. had the highest resistance to penicillin G (100%), then sulphamethoxazole/trimethoprim (28.1%). However, all *Pseudomonas* spp. isolates were 100% susceptible to ceftazidime, ciprofloxacin, amikacin, gentamicin, and imipenem. Multidrug-resistance patterns were not observed among these isolates. In this study, *Pseudomonas* spp., exhibiting spoilage features, were isolated mainly from cheeses. Isolation of this organism from processed milk highlights the need to improve the hygienic practices. All of the stages in the milk processing chain during manufacturing have to be under control to achieve the quality and safety of dairy products.

Key words: *Pseudomonas* spp., cheese, spoilage potential, antimicrobial susceptibility

INTRODUCTION

Food spoilage is a serious global problem, especially in developing countries, because of inadequate processing and refrigeration facilities (Bhunias, 2008). Undesirable microorganisms are responsible for the spoilage of dairy products, and pathogens introduced into milk and milk products by unsatisfactory milk production practices, failures in processing systems, or unsanitary practices are of primary concern (Richter and Vedamuthu, 2001). Because of the high nutritional value, water content and near neutral pH of milk, many spoilage and pathogenic microorganisms can grow in it (Ray, 2004). The predominant gram-negative bacteria such as *Pseudomonas* spp. may cause spoilage of dairy products (Cousin et al., 2001) due to the production of heat-stable extracellular enzymes (Herrera, 2001). During the pasteurization and UHT treatments many of these enzymes can survive (Ray, 2004; Bhunia, 2008). These can also be detrimental to the quality of cheese by causing bitter or rancid flavors and by impairing the coagulation properties of the milk (Richter and Vedamuthu, 2001). Proteases degrade casein, resulting in bitterness in milk, gelation of UHT-sterilized milk and decreased yields of soft cheese. Lipases catalyze the hydrolysis of triglycerides to free fatty acids and glycerol, which produce flavor defects. Lecithinases are able to disrupt milk fat globule membranes, increasing the susceptibility of milk fat to the action of lipases (Herrera, 2001; Ray, 2004).

Pseudomonas spp. are aerobic, non-spore-forming, gram-negative, rod-shaped bacteria. Some of the species show distinguishable colony morphologies or pigmentation (i.e., the blue-green derivative of phenazine, pyocyanin, and the yellow-green fluorescing pigments; Kiska and Gilligan, 1999). Psychrotrophic pseudomonads are recognized as major spoilage microorganisms based on their extracellular enzymes. The enzymes are generally good indicator of the keeping quality of protein and lipid-rich foods (Cousin et al., 2001). Although microbial spoilage may merely lead to foodstuffs being rendered unpalatable, it can also result in serious and even fatal illness (Hogg, 2005). The *Pseudomonas fluorescens* group frequently causes the spoilage of dairy

Received July 2, 2011.

Accepted September 3, 2011.

¹Corresponding author: arslan_s3@ibu.edu.tr

products. It may gel UHT milk if this is stored above 5°C. It is also important in patients with burns and cystic fibrosis (Collins et al., 1989; Sorhaug and Stepaniak, 1997). *Pseudomonas aeruginosa* causes spoilage of foods including blue milk and is pathogenic for humans and animals, often as a secondary infection (Collins et al., 1989). It is now recognized as a common source of many community acquired and nosocomial infections, and affects primarily immunocompromised people and those suffering from cystic fibrosis. The most common infections involve the cornea, skin, urinary tract, and respiratory tract, although infections may occur in essentially all anatomical locations (Yahr and Parsek, 2006). *Pseudomonas alcaligenes* and *Pseudomonas pseudoalcaligenes* are ubiquitous, and are opportunist pathogens (Collins et al., 1989).

Many aerobic pseudomonads are resistant to several antibacterial agents (Moore et al. 2006). This subject is of particular medical importance because members of this group, such as *P. aeruginosa*, are serious opportunistic human pathogens (Yahr and Parsek, 2006). The effective antimicrobial agents for the treatment of *P. aeruginosa* infections include some β -lactams, such as carbenicillin, ticarcillin, third-generation cephalosporins, the synthetic monocyclic β -lactam aztreonam, carbapenems, the aminoglycosides, and the quinolones. *Pseudomonas aeruginosa* and other fluorescent *Pseudomonas* species are in general resistant to β -lactams (Garritty et al., 2005).

The objectives of the present study were (1) to isolate *Pseudomonas* spp. from homemade cheese samples collected from the open-air public market; (2) to identify the isolates using biochemical tests; (3) to determine the extracellular enzyme and slime production, and the β -lactamase activity; and (4) to examine the antimicrobial susceptibility of *Pseudomonas* spp.

MATERIALS AND METHODS

Sample Collection

Approximately 140 samples of homemade white cheeses were randomly collected from the open-air public bazaar over a 6-mo period in Bolu (northwest Turkey). White cheese, a semisoft brined cheese, was produced by traditional methods, frequently from raw cow milk without starter cultures in rural areas. The samples were transported to the laboratory in sterile bags to minimize the possibility of contamination under cold conditions (at 4°C) and analyzed immediately.

Isolation and Identification of *Pseudomonas* spp.

A volume of 225 mL of trisodium citrate was added to the stomacher bags, each of them includes 25 g of

sample, and the samples were homogenized for 2 min by stomacher (BagMixer 400; Interscience, Paris, France). Ten-fold serial dilutions were plated onto cetrimide-Fucidin-cephaloridine-supplemented *Pseudomonas* agar base (Oxoid Ltd., Basingstoke, UK) plates (ISO-13720:2000). They were incubated at 25°C for 24 to 48 h. From each sample, several colonies were randomly chosen and subcultured for biochemical tests on nutrient agar (Merck KGaA, Darmstadt, Germany) plates. Colonies on nutrient agar are usually large (2–4 mm), flat, spreading, and pigmented. A greenish-yellow or bluish yellow fluorescent pigment may diffuse into the medium. A brown pigment is formed around the colonies (Collins et al., 1989). The presumptive *Pseudomonas* colonies were identified and confirmed by the following tests: Gram staining, motility, oxidase production, pigment production, ability to grow at 42°C, gelatin hydrolysis, tryptophan hydrolysis (indol production), utilization of carbohydrates (xylose, trehalose, glucose, arabinose, mannitol, galactose, fructose, and sucrose), and nitrate reduction (Garritty et al., 2005).

Screening of Extracellular Enzyme Producing *Pseudomonas* spp.

To determine production of proteases, lipases, and lecithinases, *Pseudomonas* isolates were plated on agar plates containing the appropriate substrates as described below. Production of proteolytic enzymes was examined on plate count agar (Merck KGaA) containing 10% skim milk powder. After incubation at 37°C for 72 h, plates were flooded with 1 N HCl to observe clearance zones (Vermelho et al., 1996). Lipase producers were screened using tributyrin agar (Merck KGaA) plates. These plates were incubated at 30°C for 48 h. The activity of the lipase was observed as a zone of hydrolysis around the bacterial colonies (Meghwanshi et al., 2006). Assessment of the production of lecithinase was performed on plate count agar (Merck KGaA) containing 10% egg yolk emulsion (Oxoid Ltd.). After incubation at 37°C for 72 h, plates were observed the presence of colonies surrounded by opaque zones (Bates and Liu, 1963).

Slime Production

Slime production of all isolates was evaluated by the Congo red agar method (Freeman et al., 1989). In the Congo red test, the medium was prepared with 37 g/L of brain heart infusion broth (Oxoid Ltd.), 50 g/L of sucrose, 10 g/L of agar, and 0.8 g/L of Congo red. Congo red stain was prepared as a concentrated aqueous solution and autoclaved at 121°C for 15 min and separately from the other medium constituents, and

was then added when the agar had cooled to 55°C. Plates were inoculated and incubated at 37°C for 24 h. A positive result was indicated by black colonies on the surface. Non-slime-producing strains developed red colonies.

***β*-Lactamase Testing**

β-Lactamase strips (Fluka Chemie AG, Buchs, Switzerland) for the acidimetric detection of the *β*-lactamase activity of all *Pseudomonas* spp. were used. The test strips were inserted into test tubes with prepared bacterial suspension in saline. A positive result was indicated by the appearance of a yellow color within a time limit (10–15 min). Strips with unchanged red color were considered as negative.

Antimicrobial Susceptibility Testing

Susceptibility to antimicrobial agents was tested using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI, 2007) guidelines. Isolates were inoculated in Mueller-Hinton broth (Merck KGaA) and adjusted to 0.5 McFarland's standard before testing, and then they were plated on Mueller-Hinton agar (Merck KGaA) plates. The following 9 different antimicrobial sensitivity discs (Oxoid Ltd.) were placed on each plate and examined against *Pseudomonas* isolates recovered from cheese samples: *β*-lactams (penicillin G, 10 U; piperacillin, 100 µg; and piperacillin/tazobactam, 110 µg), other *β*-lactams (ceftazidime, 30 µg), aminoglycosides (amikacin, 30 µg and gentamicin, 10 µg), quinolones (ciprofloxacin, 5 µg), and other antimicrobial agents including imipenem (10 µg) and sulphamethoxazole/trimethoprim (25 µg). Plates were incubated at 37°C for 24 h. The diameters of inhibition zones were recorded to the nearest millimeter and classified as susceptible, intermediate, or resistant.

RESULTS

Identification and Biochemical Characteristics of the Isolates

Thirty-two (22.9%) bacterial isolates were obtained from 140 homemade white cheese samples. The predominant isolate was *P. pseudoalcaligenes* (15%) followed by *P. alcaligenes* (5%), *P. aeruginosa* (1.4%), *P. fluorescens* biovar V (0.7%), and *P. pseudoalcaligenes* subspecies *citrulli* (0.7%; Table 1). All *Pseudomonas* spp. were actively motile after the 24-h incubation and gave oxidase-positive reaction. They did not reduce nitrate to nitrite and did not hydrolyze tryptophan.

All isolates did not produce acid from trehalose, except *P. fluorescens* biovar V; however, acid production from other carbohydrates was variable. In addition, all isolates hydrolyzed gelatin, and were able to grow at 42°C.

Production of Extracellular Enzyme, Slime, and *β*-Lactamase

The most frequent extracellular protease, lipase and lecithinase activities were as follows for *P. aeruginosa*, *P. fluorescens* biovar V, *P. pseudoalcaligenes*, *P. alcaligenes*, and *P. pseudoalcaligenes* ssp. *citrulli* isolates, respectively: protease and lecithinase production (100%), and lipase activity (100, 100, 85.7, and 42.9%, and nonlipolytic, respectively). None of the isolated *Pseudomonas* spp. produced slime, which is the indicator of pathogenicity. Nevertheless, *Pseudomonas* isolates did not show *β*-lactamase activity. The results of production of the extracellular enzyme, slime, and *β*-lactamase for each *Pseudomonas* spp. isolated from homemade white cheese samples are shown in Table 1.

Antimicrobial Resistance Profiles

The results of antimicrobial susceptibility of *Pseudomonas* spp. isolated from cheese are shown in Table 2. According to this, *Pseudomonas* spp. had the highest resistance to penicillin G (100%), then sulphamethoxazole/trimethoprim (28.1%). However, all *Pseudomonas* spp. isolates were 100% susceptible to ceftazidime, ciprofloxacin, amikacin, gentamicin, and imipenem. Multidrug-resistance patterns were not observed among these isolates.

DISCUSSION

Pseudomonas spp. isolated from homemade white cheese samples were well-characterized in this study on the basis of several biochemical characteristics. The isolates of *Pseudomonas* spp. were detected in 22.9% of the samples. One of the microbiological components of raw milk can be psychrotrophic microorganisms, mainly belonging to the genus *Pseudomonas*, which are responsible of the spoilage of milk and dairy products at low temperature (Ray, 2004).

Reported previous studies were focused on isolation of *Pseudomonas* spp. from raw milk samples and dairy products. Even though various *Pseudomonas* spp. have been isolated from raw milk samples, the *P. fluorescens* group is the most frequently involved in dairy products by the depredation of milk (Wiedmann et al., 2000; Dogan and Boor, 2003). Gennari and Dragotto (1992) reported that prevalence of *Pseudomonas* spp. was 86% from the foodstuffs and environmental samples, and

Table 1. Characteristics of *Pseudomonas* spp. isolates

Strain	Identification	Pigmentation	Protease activity	Lipase activity	Lecithinase activity	Slime production	β -Lactamase
3/PS1	<i>Pseudomonas pseudoalcaligenes</i>	Light brown	+	+	+	–	–
5/PS1	<i>P. pseudoalcaligenes</i>	Light brown-yellow	+	+	+	–	–
6/PS2	<i>P. pseudoalcaligenes</i>	Yellow	+	+	+	–	–
7/PS1	<i>P. pseudoalcaligenes</i>	Cream	+	+	+	–	–
9/PS1	<i>P. pseudoalcaligenes</i>	Yellow	+	+	+	–	–
13/PS1	<i>Pseudomonas alcaligenes</i>	Light brown	+	+	+	–	–
18/PS2	<i>P. pseudoalcaligenes</i>	Light brown	+	+	+	–	–
24/PS1	<i>P. pseudoalcaligenes</i>	Light brown	+	+	+	–	–
39/PS2	<i>P. alcaligenes</i>	Light brown	+	+	+	–	–
40/PS1	<i>P. pseudoalcaligenes</i>	Light brown	+	+	+	–	–
43/PS2	<i>P. pseudoalcaligenes</i>	Light green	+	+	+	–	–
52/PS1	<i>P. pseudoalcaligenes</i>	Light brown	+	+	+	–	–
55/PS1	<i>P. alcaligenes</i>	Yellow	+	+	+	–	–
64/PS1	<i>P. alcaligenes</i>	Yellow	+	–	+	–	–
68/PS2	<i>Pseudomonas aeruginosa</i>	Light brown	+	+	+	–	–
69/PS1	<i>P. pseudoalcaligenes</i>	Yellow	+	–	+	–	–
71/PS1	<i>P. pseudoalcaligenes</i>	Yellow	+	+	+	–	–
73/PS1	<i>P. pseudoalcaligenes</i>	Light brown	+	+	+	–	–
77/PS1	<i>P. pseudoalcaligenes</i>	Light brown	+	+	+	–	–
78/PS1	<i>P. pseudoalcaligenes</i>	Light brown	+	+	+	–	–
80/PS2	<i>Pseudomonas fluorescens</i> biovar V	Light brown	+	+	+	–	–
87/PS1	<i>P. pseudoalcaligenes</i>	Yellow	+	+	+	–	–
96/PS1	<i>P. pseudoalcaligenes</i>	Yellow	+	–	+	–	–
98/PS2	<i>P. alcaligenes</i>	Cream	+	–	+	–	–
101/PS1	<i>P. alcaligenes</i>	Cream	+	–	+	–	–
102/PS2	<i>P. pseudoalcaligenes</i> subspecies <i>citrulli</i>	Cream	+	–	+	–	–
105/PS2	<i>P. pseudoalcaligenes</i>	Green-yellow	+	+	+	–	–
106/PS2	<i>P. pseudoalcaligenes</i>	Green-yellow	+	+	+	–	–
126/PS1	<i>P. alcaligenes</i>	Cream	+	–	+	–	–
129/PS2	<i>P. pseudoalcaligenes</i>	Light green	+	–	+	–	–
137/PS4	<i>P. aeruginosa</i>	Cream	+	+	+	–	–
140/PS1	<i>P. pseudoalcaligenes</i>	Green-yellow	+	+	+	–	–

Table 2. Antimicrobial susceptibility of 32 isolates of *Pseudomonas* spp.¹

Antimicrobial agent	Concentration (µg/disc)	Resistant	Intermediate	Susceptible
Piperacillin	100	0	2 (6.2)	30 (93.8)
Piperacillin/tazobactam	110	0	2 (6.2)	30 (93.8)
Penicillin G (U)	10	32 (100)	0	0
Amikacin	30	0	0	32 (100)
Gentamicin	10	0	0	32 (100)
Ciprofloxacin	5	0	0	32 (100)
Ceftazidime	30	0	0	32 (100)
Imipenem	10	0	0	32 (100)
Sulfamethoxazole/trimethoprim	25	9 (28.1)	19 (59.4)	4 (12.5)

¹Results are given as numbers (percents in parentheses).

Pseudomonas fluorescens biovar V was most frequently isolated (38.3%). Besides, 54.5% of *Pseudomonas* spp. were revealed from raw milk cheeses (Leriché et al., 2004), and 50% of *Pseudomonas* spp. were obtained from 1-d-old raw milk cheeses (Morales et al., 2005). In a previous study, the incidence of *Pseudomonas* spp. was 30.5% from raw milk samples (Uraz and Çıtak, 1998). In addition, 67% of *Pseudomonas* spp. in UHT milk was found by Chen et al. (2011). *Pseudomonas pseudoalcaligenes* and *P. alcaligenes* are encountered rarely in clinical and environmental samples (Kiska and Gilligan, 1999); however, the current study revealed the isolation of *P. pseudoalcaligenes* and *P. alcaligenes* from cheese samples.

In the present study, most of the isolated *Pseudomonas* spp. were found to produce extracellular enzymes: protease, lipase, and lecithinase. The results of enzyme activity were agreement with Wiedmann et al. (2000), Dogan and Boor, (2003), and Munsch-Alatossava and Alatossava (2006). These enzymatic activities are very important for the dairy industry. Lipase, protease, and lecithinase are potentially able to cause spoilage of the dairy products (Ray, 2004). Such enzymes can degrade the carbohydrates, proteins, and fats of milk, leading to production of noxious end products and, in some cases, to decrease in cheese yield (Richter and Vedomuthu, 2001; Ray, 2004). Several groups of organisms contain members potentially contributing to milk spoilage under certain conditions. This makes the microbial ecology of raw milk and raw milk cheeses very complex and, thus, spoilage is difficult to prevent. Moreover, a need still exists to assess, within each spoilage group, which species can more often occur and affect the quality of raw milk and raw milk cheese (Ercolini et al., 2009).

Bacterial antimicrobial resistance has become a global problem in both the medical and agricultural fields. Antibiotic resistant strains of bacteria are an increasing threat to animal and human health, with resistance mechanisms determined for all known antimicrobials currently available for clinical use in human

and veterinary medicine (McDermott et al., 2002). Most *Pseudomonas* spp. are naturally resistant to penicillin and the majority of related β -lactam antibiotics (Ryan and Ray, 2004). In our study, all *Pseudomonas* spp. isolated from homemade cheeses were resistant to penicillin G and sulphamethoxazole/trimethoprim (28.1%). Therefore, *Pseudomonas* spp. had intermediate resistance to piperacillin and piperacillin/tazobactam (6.2%), which are β -lactams, whereas they did not have any resistance to ceftazidime, one other β -lactam. A paucity of knowledge exists of antimicrobial susceptibility profiles of food-related bacteria, even though antimicrobial sensitivities of clinical *Pseudomonas* spp., especially *P. aeruginosa* have been well studied. Clinical studies were emphasized that *P. aeruginosa* was resistant to more than 1 antibiotic (Corona-Nakamura et al., 2001; Hsueh et al., 2005; Muthu et al., 2006). In a study from Finland regarding antibiotic resistance of raw milk-associated *Pseudomonas* spp., the results of the antimicrobial susceptibility of the isolates were considerably similar to our results. The *Pseudomonas* spp. isolates almost all were sensitive to piperacillin, piperacillin/tazobactam, ciprofloxacin, amikacin, and gentamicin, but susceptibility level of other antimicrobial agents such as ceftazidime and imipenem was variable (Munsch-Alatossava and Alatossava, 2007). Jensen et al., (2001) also found *Pseudomonas* spp. isolated from environmental samples had no resistance to ciprofloxacin and gentamicin, like our findings.

CONCLUSIONS

In conclusion, in the present study, *Pseudomonas* spp. exhibiting spoilage features were isolated mainly from cheeses. The results obtained regarding isolation of this organism from processed milk highlights the need to improve hygienic practices. This type of organism, found in raw milk cheeses, may depend on inadequate pasteurization of milk or use of unpasteurized milk, the extent of cleaning and sanitizing of milking equipment

and utensils, and the time and temperature of storage. All of the stages in the milk processing chain during manufacturing have to be under control to achieve the quality and safety of dairy products.

REFERENCES

- Bates, J. L., and P. V. Liu. 1963. Complementation of lecithinase activities by closely related pseudomonads: Its taxonomic implication. *J. Bacteriol.* 86:585–592.
- Bhunia, A. K. 2008. Foodborne Microbial Pathogens: Mechanisms and Pathogenesis. Springer, West Lafayette, IN.
- Chen, T. R., Q. K. Wei, and Y. J. Chen. 2011. *Pseudomonas* spp. and *Hafnia alvei* growth in UHT milk at cold storage. *Food Contr.* 22:697–701.
- CLSI (Clinical and Laboratory Standards Institute). 2007. Performance Standards for Antimicrobial Susceptibility Testing: Seventeenth Informational Supplement. CLSI document M100–S17. CLSI, Wayne, Pennsylvania, PA.
- Collins, C. H., P. M. Lyne, and J. M. Grange. 1989. Collins and Lyne's Microbiological Methods (*Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Flavobacterium*, *Chromobacterium* and *Acetobacter*). 6th ed. Butterworth-Heinemann, Oxford, UK.
- Corona-Nakamura, A. L., M. G. Miranda-Novales, B. Leños-Miranda, L. Portillo-Gómez, A. Hernandez-Chavez, J. Anthor-Rendón, and S. Aguilar-Benavides. 2001. Epidemiologic study of *P. aeruginosa* in critical patients and reservoirs. *Arch. Med. Res.* 32:238–242.
- Cousin, M. A., J. M. Jay, and P. C. Vasavada. 2001. Psychrotrophic microorganisms. Pages 159–166 in Compendium of Methods for the Microbiological Examination of Foods. F. P. Downes and K. Ito, K. ed. American Public Health Association, Washington, DC.
- Dogan, B., and K. J. Boor. 2003. Genetic diversity and spoilage potentials among *Pseudomonas* spp. isolated from fluid milk products and dairy processing plants. *Appl. Environ. Microbiol.* 69:130–138.
- Ercolini, D., F. Russo, I. Ferrocino, and F. Villani. 2009. Molecular identification of mesophilic and psychrotrophic bacteria from raw cow's milk. *Food Microbiol.* 26:228–231.
- Freeman, D. J., F. R. Falkner, and C. T. Keane. 1989. New method for detecting slime production by coagulase negative staphylococci. *J. Clin. Pathol.* 42:872–874.
- Garrity, G. M., J. A. Bell, and T. Lilburn. 2005. Order IX. Pseudomonadales. Pages 323–371 in Bergey's Manual of Systematic Bacteriology. Vol. 2. D. J. Brenner, N. R. Krieg, and G. M. Garrity, ed. Springer, New York, NY.
- Gennari, M., and F. Dragotto. 1992. A study of the incidence of different fluorescent *Pseudomonas* species and biovars in the microflora of fresh and spoiled meat and fish, raw milk, cheese, soil and water. *J. Appl. Bacteriol.* 72:281–288.
- Herrera, A. G. 2001. Psychrotrophic microorganisms. Pages 3–11 in Food Microbiology Protocols. J. F. T. Spencer and de A. L. R. Spencer, ed. Humana Press Inc., Totowa, New Jersey, NJ.
- Hogg, S. 2005. Essential Microbiology (Microorganisms in Industry). John Wiley and Sons, West Sussex, UK.
- Hsueh, P.-R., W.-H. Chen, and K.-T. Luh. 2005. Relationship between antimicrobial use and antimicrobial resistance in gram-negative bacteria causing nosocomial infections from 1991–2003 at a university hospital in Taiwan. *Int. J. Antimicrob. Agents* 26:463–472.
- Jensen, L. B., S. Baloda, M. Boye, and F. M. Aarestrup. 2001. Antimicrobial resistance among *Pseudomonas* spp. and *Bacillus cereus* group isolated from Danish agricultural soil. *Environ. Int.* 26:581–587.
- Kiska, D. L., and P. H. Gilligan. 1999. *Pseudomonas*. Pages 517–525 in Manual of Clinical Microbiology. P. R. Murray, E. J. Baron, M. A. Tenover, F. C. Tenover, and R. H. Tenover, ed. American Society for Microbiology Press, Washington, DC.
- Lerliche, F., A. Bordessoules, K. Fayolle, R. Karoui, K. Laval, L. Leblanc, and E. Dufour. 2004. Alteration of raw-milk cheese by *Pseudomonas* spp.: Monitoring the sources of contamination using fluorescence spectroscopy and metabolic profiling. *J. Microbiol. Methods* 59:33–41.
- McDermott, P. F., S. Zhao, D. D. Wagner, S. Simjee, R. D. Walker, and D. G. White. 2002. The food safety perspective of antibiotic resistance. *Anim. Biotechnol.* 13:71–84.
- Meghwanshi, G. K., L. Agarwal, K. Dutt, and R. K. Saxena. 2006. Characterization of 1,3-regiospecific lipases from new *Pseudomonas* and *Bacillus* isolates. *J. Mol. Catal., B Enzym.* 40:127–131.
- Moore, E. R. B., B. J. Tindall, V. A. P. M. Dos Santos, D. H. Pieper, J. Ramos, and N. J. Palleroni. 2006. Nonmedical: *Pseudomonas*. Pages 646–703 in The Prokaryotes. Vol. 6. M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, and E. Stackebrandt, ed. Springer, New York, NY.
- Morales, P., E. Fernandez-Garcia, and M. Nunez. 2005. Volatile compounds produced in cheese by *Pseudomonas* strains of dairy origin belonging to six different species. *J. Agric. Food Chem.* 53:6835–6843.
- Munsch-Alatossava, P., and T. Alatossava. 2006. Phenotyping characterization of raw milk-associated psychrotrophic bacteria. *Microbiol. Res.* 161:334–346.
- Munsch-Alatossava, P., and T. Alatossava. 2007. Antibiotic resistance of raw-milk-associated psychrotrophic bacteria. *Microbiol. Res.* 162:115–123.
- Muthu, S. E., R. A. Aberna, V. Mohan, G. Premalatha, R. S. Srinivasan, S. P. Thyagarajan, and U. A. Rao. 2006. Phenotypes of isolates of *P. aeruginosa* in a diabetes care center. *Arch. Med. Res.* 37:95–101.
- Ray, B. 2004. Fundamental Food Microbiology. CRC Press, Boca Raton, FL.
- Richter, R. L., and E. R. Vedamuthu. 2001. Milk and Milk Products. Pages 483–495 in Compendium of Methods for the Microbiological Examination of Foods. F. P. Downes and K. Ito, ed. American Public Health Association, Washington, DC.
- Ryan, K. J., and C. G. Ray. 2004. Sherris Medical Microbiology. 4th ed. McGraw Hill, New York, NY.
- Sorhaug, T., and L. Stepaniak. 1997. Psychrotrophs and their enzymes in milk and dairy products: Quality aspects. *Trends Food Sci. Technol.* 8:35–41.
- Uraz, G., and S. Çitak. 1998. The isolation of *Pseudomonas* and other gram(–) psychrotrophic bacteria in raw milks. *J. Basic Microbiol.* 38:129–134.
- Vermelho, A. B., M. N. L. Meirelles, A. Lopes, S. D. G. Petinate, A. A. Chaia, and M. H. Branquinho. 1996. Detection of extracellular proteases from microorganisms on agar plates. *Mem. Inst. Oswaldo Cruz* 91:755–760.
- Wiedmann, M., D. Weilmeier, S. S. Dineen, R. Ralyea, and K. J. Boor. 2000. Molecular and phenotypic characterization of *Pseudomonas* spp. isolated from milk. *Appl. Environ. Microb.* 66:2085–2095.
- Yahr, T. L., and M. R. Parsek. 2006. *Pseudomonas aeruginosa*. Pages 704–713 in The Prokaryotes. Vol. 6. M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, and E. Stackebrandt, ed. Springer, New York, NY.