When cheese gets the blues: *Pseudomonas fluorescens* as the causative agent of cheese spoilage

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ABSTRACT

A bacterial contamination of fresh, low-acid cheese that resulted in production of a blue fluorescent pigment on the surface of the cheese was determined to be caused by *Pseudomonas fluorescens* biovar IV, a gram-negative bacteria that produces a blue, nondiffusible pigment as well as the soluble pigment pyoverdine, which fluoresces under UV light. Ten isolates collected from contaminated cheese and environmental samples were initially identified as *P. fluorescens* using 16S rDNA sequencing, but only 8 of the isolates produced blue pigment and fluoresced under UV light when re-inoculated onto fresh, low-acid cheese. The Biolog Metabolic Fingerprint system (Biolog Inc., Hayward, CA) and the Analytical Profile Index (BioMerieux Vitek Inc., Hazelwood, MO) for nonenteric gram-negative species as well as *EcoRI* ribotyping did not differentiate between the isolates that produced blue color and those that did not. Pulsed field gel electrophoresis with the enzyme *XbaI* was able to distinguish between the isolates that produced pigment and those that did not and allowed for identification of a specific environmental site (i.e., an overhead cheese vat agitator system) as the likely source of product contamination.

Key words: blue spoilage, Queso Fresco, subtyping tools

INTRODUCTION

Food loss attributed to microbial spoilage is a major concern in the food industry. Microbial contamination and growth can result in undesirable changes in appearance, texture, flavor, and odor that reduce food quality (Sperber and Doyle, 2009). *Pseudomonas* spp. are among the most common microorganisms implicated in food spoilage and are particularly important in refrigerated foods because many strains are psychrotolerant (Kraft,

1992). Improving identification and tracking methods for these organisms in food production and processing systems is important in understanding and reducing the frequency of contamination, as well as eliminating niche populations in food processing systems such that contamination of food products can be prevented.

This paper describes a unique spoilage pattern, characterized by a distinct blue discoloration of a Latin-style fresh cheese (Queso Fresco), which occurred in a commercial plant manufacturing different Latin-style fresh cheeses. Although not detected in every day's production, this spoilage persisted over a 9-mo period, suggesting a persistent reservoir of the causative organism. A combination of plant investigations, traditional microbiological methods, and molecular subtyping methods were used to characterize the causative agent for this unusual spoilage pattern as a specific *Pseudomonas fluorescens* strain and to identify the likely source of this spoilage organism.

MATERIALS AND METHODS

Description of Cheese Manufacturing and Plant Characteristics

The plant that experienced the spoilage issue detailed here manufactures Latin-style fresh cheeses. Cheeses were manufactured at the processing facility by heating pasteurized milk, filling 2 cheese vats (CV-1, CV-2) with milk at approximately 35°C (95°F), and adding rennet and acidulant to form the curd. The curd was then broken with agitation, pumped onto a press table (PT), raked and pressed to remove whey, milled with added salt, molded, and packaged. Target salt concentration was 2.7% and final pH was typically 5.9 to 6.0.

Sample Collection

Samples were collected at the plant on 3 occasions over a 4-mo period. The initial samples tested (sampling 1) included 2 samples of cheese curd that were collected after the pressing step and exhibited the blue

Received February 26, 2011.

Accepted March 6, 2011.

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pigment defect after 48 to 72 h of incubation at room temperature. These 2 samples were from 2 separate processing days and represented cheese curd pressed on 2 press tables (**PT-1**, **PT-2**).

The second set of samples collected (sampling 2) included samples collected during various stages of manufacturing as well as swabs from selected environmental sites. Samples collected representing different process steps for cheese made from 2 cheese vats (**CV-1**, **CV-2**) included (1) pasteurized milk as it was pumped into the cheese vat; (2) curd and whey after breaking the rennet-set milk; (3) curd and whey as it was pumped to the drain and press table; (4) curd after draining before pressing; and (5) curd after pressing. All of these samples were collected for both vats. The packaged products made from these vats were also collected along with samples of rennet diluted with pasteurized water and water from hoses used for clean up in the cheese vat and press table areas.

Surface swab samples from sampling 2 were collected from (1) the drain holes of the cheese vat agitator track (housing for an automated agitator system that is located above and moves the length of the cheese vat); (2) the drain tubes of the cheese vat agitator track; (3) the cheese vat agitator track guard; (4) cheese vat agitator paddles (as cracks were observed there); (5) the rennet bucket; (6) the press table hydraulic piston shafts; and (7) the press table room refrigeration unit grill.

The third set of samples (sampling 3) was collected based on results from sampling 2 and included swabs of the agitator drain tubes, agitator track, and agitator paddles for both **CV-1** and **CV-2**.

Bacteriological Analyses

Unless otherwise noted, all samples were assessed for standard plate count, gram-negative bacteria count on crystal violet tetrazolium agar (**CVTA**), and coliform bacteria count on violet red bile agar according to *Standard Methods for the Examination of Dairy Products* (Frank and Yousef, 2004); all media were obtained from Difco, BD Diagnostics (Franklin Lakes, NJ). Incubations for all counts were performed in accordance with Frank and Yousef (2004).

Cheese curd samples exhibiting blue pigment, equipment surface swabs, water, and rennet were tested upon arrival. Milk samples were plated upon arrival to the laboratory and after storage for 24 h at 21°C or 14 d at 6°C. Cheese curd samples collected from the cheese vats and the press table were held for 72 h at room temperature and for 21 d at 6°C and were only tested if blue pigment production was observed. The final packaged cheese products were tested initially and after 21 d at 6°C. Water and the diluted rennet sample were plated

both undiluted and after mixing 20 mL with 80 mL of UHT milk, followed by incubation for 14 d at 6°C.

Isolate Collection

Bacterial isolates were collected from SPC and CVTA plates from each of the 2 cheese curd samples that exhibited the blue pigment defect (after incubation at room temperature), the rennet sample (before and after incubation in UHT milk), and selected environmental swabs. Colonies representing each visually distinct morphology present on the plates described above were selected and streaked for purity on brain heart infusion agar (**BHI**). Gram stain, catalase, and oxidase tests were performed on the isolates before they were placed in frozen storage at −80°C in 15% glycerol. For further characterization, isolates were taken from storage, streaked on BHI agar, and incubated at 32°C unless otherwise noted. Additional information on isolates collected in this study as well as 16S rDNA sequences and ribotype images can be found at www.pathogentracker.net.

16S rDNA Sequencing

Sequencing of the 3' end of a 616-nucleotide 16S rDNA fragment, as described previously by Huck et al. (2007), was used to characterize each isolate. The 16S sequence data were used to characterize isolates to the genus and species level using the Ribosomal Database Project (RDP) database (<http://rdp.cme.msu.edu/index.jsp>).

Biolog

The Biolog GN2 MicroPlate system (Biolog Inc., Hayward, CA) was used following the manufacturer's Gram Negative Non-Enteric (GN-NENT) protocol. Cultures were streaked onto BHI agar and incubated for 18 h at 32°C. A uniform suspension (transmittance level of 52%) of each isolate was prepared in Gram Negative/Gram Positive-Inoculating Fluid (GN/GP-IF). Suspensions were inoculated into the MicroPlates, incubated at 32°C for 18 h, and read using the MicroLog 3 system.

Analytical Profile Index

The Analytical Profile Index (**API**) 20NE test kit (BioMerieux Vitek Inc., Hazelwood, MO) was used according to the manufacturer's instructions. Examination of the strips was conducted after 24 and 48 h. Identification of the isolates was performed according to the API 20NE identification instructions online (<http://apiweb.biomerieux.com>).

Pulsed Field Gel Electrophoresis

Pulsed field gel electrophoresis (PFGE) of all isolates was performed using the standard Centers for Disease Control and Prevention PulseNet protocol for enteric pathogens (Ribot et al., 2006). Briefly, isolates were grown on BHI agar plates at 32°C for 18 h. Bacterial cultures were embedded in 1% agarose plugs (SeaKem Gold agarose, Cambrex, Rockland, ME), lysed, washed, and digested with the restriction enzyme *Xba*I for 2 h at 37°C. Size separation of restricted DNA fragments was performed for 20 to 22 h in 1% agarose gels by using a contour clamped homogeneous electrical field Mapper XA (Bio-Rad Laboratories, Hercules, CA); voltage was set at 6 V/cm with switch times of 4 s to 40.01 s. The *Xba*I-digested *Salmonella enterica* serotype Braenderup (CDCH9812) DNA was used as a reference size standard. Pattern images were captured with a Bio-Rad Gel Doc and the Multi Analyst software version 1.1 (Bio-Rad Laboratories). The PFGE patterns were analyzed and compared using the BioNumerics version 3.5 software (Applied Maths, Sint-Martens-Latem, Belgium).

Ribotyping

Automated ribotyping was performed using the RiboPrinter Microbial Characterization System (DuPont Qualicon, Wilmington, DE) and the procedure described by Bruce (1996) and reported by Wiedmann et al. (1997). In brief, this automated process includes bacterial cell lysis and cleavage of the DNA using the restriction enzyme *Eco*RI. Then, the DNA fragments are separated by size using electrophoresis and analyzed using a modified Southern hybridization blotting technique. The DNA is hybridized with a labeled rRNA operon probe derived from *Escherichia coli* and the bands are detected using a chemiluminescent substrate. An image, captured using a customized charge-coupled device camera, is electronically transferred to the system's computer. Each sample lane of data is normalized to a standard marker set, characterized, and identified using similarity measurements to previously run isolates and reference patterns.

Confirmation of Pigment Production

To determine if a given isolate was able to produce blue pigment, selected isolates were inoculated onto fresh cheese and streaked on potato dextrose agar (PDA; Difco, BD Diagnostics; Palleroni, 1984). Each isolate was grown on BHI agar plates for 24 h at 32°C, and approximately 10^5 cfu was inoculated from the plate onto a separate piece of fresh, rennet-set cheese

curd made from pasteurized milk. Inoculated cheese and corresponding uninoculated controls were stored at 6°C in covered Petri dishes for 48 h. Additionally, isolated colonies from the BHI plates were streaked onto PDA and incubated at 32°C for 48 h. After respective incubation periods, both the inoculated cheese and the PDA were observed for growth and pigment production. The PDA plates were also observed under a UV light for fluorescence.

RESULTS

Classical Microbiological Analyses Identify High Bacterial Counts in Cheese and Selected Environmental Sites

In the initial sampling, 2 cheese curd samples were collected, both of which exhibited the blue pigment defect after 48 h of incubation at room temperature; both samples had SPC and CVTA counts $>1 \times 10^7$ cfu/g. These 2 samples represented curds collected after pressing and draining but before molding and packaging, suggesting that contamination occurred before or during the pressing and draining step. In the second sampling, none of the cheese curd samples ($n = 8$) collected during processing and neither of the packaged cheese from the 2 cheese vats showed blue pigment (after incubation at room temperature or refrigerated temperatures for 72 h and 21 d, respectively). These findings suggested that the organisms responsible for the blue pigment production were either uncommon or not present in the product at the second sampling, which occurred 87 d after the initial sampling (i.e., sampling 1). Despite the absence of blue pigment in the products from the second sampling, SPC and CVTA counts of this cheese curd after pressing and the packaged product were $>1 \times 10^6$ cfu/g (after storage at 6°C for 21 d), suggesting postpasteurization contamination.

Gram-negative bacteria were not detected in any of the milk samples immediately after collection, whereas SPC were at or below 1×10^2 cfu/mL. After 14 d of incubation at 6°C, the milk samples had SPC counts $>1 \times 10^6$ cfu/mL, and CVTA counts were $<1 \times 10^2$ cfu/mL. Water samples collected from the hoses in the areas where the vat and press table were located did not appear to be potential sources of contamination; SPC and CVTA counts were $<1 \times 10^1$ cfu/mL when plated directly and $<1 \times 10^2$ cfu/mL when the water was added to UHT milk, followed by incubation at 6°C for 14 d. Diluted rennet sampled from the processing facility had low CVTA counts immediately after collection, but after storage at 6°C for 14 d in UHT milk the SPC and CVTA counts exceeded 1×10^7 cfu/mL.

Surface swabs were taken from CV-1 and CV-2, PT-1, PT-2, and PT-3 (only swabbed during sampling 2), and the rennet bucket before and during production on 2 occasions (Table 1). Gram-negative bacteria were recovered, on both occasions, from the swabs of the agitator track guard above CV-1 (at levels of $>1 \times 10^5$ cfu/swab) and the swabs of the CV-2 agitator track drain tubes (at 1×10^7 cfu/swab; Table 1). Further characterization of these isolates showed that 2 of the gram-negative isolates found on the agitator track guard above CV-1 from sampling 3 were the same blue pigment-producing *P. fluorescens* responsible for the cheese spoilage. The remaining isolates collected from these 2 locations failed to produce blue pigment. Gram-negative bacteria were also present in the CV-1 drain tube (on both sampling occasions) as well as the CV-2 agitator blade (Table 1); however, isolates collected from these 2 sources failed to produce blue pigment. Swabs taken from the PT-1, PT-2, and PT-3 press shafts, CV-1 agitator blade, CV-2 agitator track, the rennet bucket, and the press room refrigeration unit did not show presence of gram-negative bacteria (Table 1).

16S Sequencing Identifies *P. fluorescens* as the Predominant Organism in Samples Linked to Cheese with Blue Color Development

Based on 16S rDNA sequencing, the principal organism in contaminated cheese curd sample PT-1 was *P. fluorescens* (Table 2). *Lactococcus lactis* was also isolated from that sample. *Pseudomonas fluorescens* with the same 16S rDNA sequence as the isolates in PT-1 was also isolated from PT-2 in addition to *L. lactis*, *Klebsiella* spp., *Pantoea* sp., and *Bacillus cereus*. All *P. fluorescens* isolates from the defective cheese curd

produced blue pigment and showed fluorescence under UV light when streaked onto PDA and inoculated on fresh, low-acid cheese (Table 3, Figure 1a, b).

Pseudomonas fluorescens was also isolated from 3 environmental swabs taken from the agitator drain on CV-1 and CV-2, as well as the CV-1 agitator track (Table 2). Four of the 11 environmental isolates identified as *P. fluorescens* had 16S rDNA sequences identical to the isolates from the contaminated cheese curd samples. These 4 isolates were also inoculated on fresh, low-acid cheese and streaked onto PDA for confirmation of pigment production. Only 2 of those 4 isolates showed production of both the blue pigment and fluorescence (Table 3) despite having the same 16S sequence as those that did not produce the pigments. Other isolates collected from environmental swabs included *L. lactis* and *Comamonas* spp., whereas isolates from the rennet sample were identified as *Pseudomonas* spp. (Table 2).

In total, 8 of the 10 isolates of *P. fluorescens* tested from this study produced blue pigment when streaked onto PDA and inoculated onto fresh cheese (Table 3). When the PDA plates and inoculated cheese were examined under UV light for fluorescence only cultures that developed blue pigment fluoresced (Table 3). Isolates that were confirmed for blue pigment and fluorescence were from the 2 defect cheese curd samples (PT-1, PT-2) and the agitator track guard swab.

PFGE Analyses Are Required for Specific Detection and Source Tracking of the *P. fluorescens* Strain that Produces Blue Pigment

Sequencing of 16S rDNA showed that *P. fluorescens* isolates that produced blue and fluorescent pigments and those that did not had identical partial 16S rDNA

Table 1. Results of SPC and crystal violet tetrazolium agar (CVTA) enumeration (log cfu/swab) from swab samples collected in the processing facility¹

Sample ²	Sampling 2		Sampling 3	
	SPC	CVTA ³	SPC	CVTA
CV-1 agitator drain tube	4.1	3.2	6.4	7.0
CV-1 agitator track	>5.5	>5.5	5.1	5.4
CV-1 agitator blade	3.7	<1.7	<2.0	<2.0
Rennet dilution bucket	3.5	<1.7		
PT-1 press shaft	<1.7	<1.7		
PT-2 press shaft	<1.7	<1.7		
PT-3 press shaft	2.8	<1.7		
CV-2 agitator drain tube			7.2	7.0
CV-2 agitator track			<2.0	<2.0
CV-2 agitator blade			3.6	3.1

¹Area swabbed was approximately 50 cm² for flat surfaces. Areas swabbed for other surfaces varied with configuration. Detection limits were 1.7 log cfu/swab and 2.0 log cfu/swab for samplings 2 and 3, respectively. Samplings 2 and 3 indicate separate sampling days; only product samples were collected during sampling 1.

²CV = cheese vat; PT = press table.

³CVTA was used to enumerate gram-negative bacteria.

Table 2. Identification, based on 16S sequence, of isolates from cheese samples (PT-1, PT-2) with blue defect, rennet sample, and cheese vat (CV-1, CV-2) agitator swab samples

Sample source (sampling) ¹	16S Identification (no. of isolates)	FSL no. ²
PT-1 cheese curd (1)	<i>Lactococcus lactis</i> (2)	W5-204, W5-205
PT-1 cheese curd (1)	<i>Pseudomonas fluorescens</i> (4)	W5-203, W5-206, W5-207, W5-208
PT-2 cheese curd (1)	<i>Bacillus cereus</i> (1)	W5-294
PT-2 cheese curd (1)	<i>Klebsiella</i> spp. (2)	W5-288, W5-290
PT-2 cheese curd (1)	<i>Lactococcus lactis</i> (4)	W5-291, W5-292, W5-293, W5-295
PT-2 cheese curd (1)	<i>Pantoea</i> sp. (1)	W5-289
PT-2 cheese curd (1)	<i>Pseudomonas fluorescens</i> (2)	W5-287, W5-296
Rennet (2)	<i>Pseudomonas</i> spp. (2)	W5-302, W5-303
Swab-agitator drain, CV-1 (2)	<i>Lactococcus lactis</i> (1)	W5-301
Swab-agitator drain, CV-1 (2)	<i>Pseudomonas fluorescens</i> (2)	W5-299, W5-300
Swab-agitator drain, CV-1 (3)	<i>Pseudomonas fluorescens</i> (3)	W5-315, W5-316, W5-317
Swab-agitator drain, CV-2 (3)	<i>Pseudomonas fluorescens</i> (3)	W5-318, W5-319, W5-320
Swab-agitator track, CV-1 (2)	<i>Comamonas</i> spp. (2)	W5-306, W5-307
Swab-agitator track, CV-1 (2)	<i>Lactococcus lactis</i> (1)	W5-308
Swab-agitator track, CV-1 (3)	<i>Pseudomonas fluorescens</i> (3)	W5-325, W5-326, W5-327

¹PT = press table; CV = cheese vat; samplings 1, 2, and 3 indicate different dates on which samples were collected.

²FSL no. = Food Safety Laboratory (Cornell University, Ithaca, NY) identification number (e.g., FSL W5-204).

sequences. Therefore, we further characterized 10 *P. fluorescens* isolates from this study (including 2 that did not form the pigment; Table 3) as well as 2 comparison *P. fluorescens* isolates that did not produce pigments, from an unrelated study (Ranieri and Boor, 2009), using different molecular subtyping and identification methods.

All isolates were confirmed as gram-negative, catalase-positive, and oxidase-positive rods. All isolates were identified as *P. fluorescens* by API and had the same API code, except for 4 of the 12 isolates that had a variable gelatinase reaction whereas the remainder had positive gelatinase reactions (this difference did not correspond to pigment production). The Biolog system was also unable to distinguish between isolates producing blue pigment producers and those not producing blue pigment. The Biolog system identified all of the isolates from this study as *P. fluorescens*, whereas the 2 isolates from the unrelated study (R5-199, R5-202) were the only isolates identified as different species (*P. tolaasii* and *P. synxantha*, respectively).

No difference was found between pigment-producing isolates and non-pigment-producing isolates when subtyped using ribotyping (Table 3). Pulsed field gel electrophoresis was the only method applied that was able to distinguish between isolates that produced pigment and those that did not. Seven of the 8 isolates that produced blue pigment had identical PFGE patterns; the other pigment-producing isolate (W5-296) had a pattern that differed from the other pigment-producing isolates by 2 bands. The 2 non-pigment-producing isolates (W5-319 and W5-327) had identical PFGE patterns that differed from all the pigment-producing strains by >10 bands (Table 3).

DISCUSSION

P. fluorescens as the Causative Agent of a Pigment Defect in Latin-Style Cheese

A variety of classical microbiological and molecular subtyping tests confirmed that a blue pigment-producing strain of *P. fluorescens* was the cause of the unique spoilage experienced by a manufacturer of Latin-style cheese. *Pseudomonas fluorescens* is a gram-negative rod with strains that are capable of producing a blue, nondiffusible pigment as well as the soluble pigment pyoverdine, which fluoresces under UV light (Palleroni, 1984). Specifically, 5 biovars of *P. fluorescens* exist, of which biovar IV is the only one that produces pyoverdine as well as the blue, nondiffusible pigment (Palleroni, 1984). The type of spoilage investigated in this study appears to be relatively rare, although *Pseudomonas* spp. have been implicated in pigment-related spoilage of other fresh cheeses, including yellow and blue pigments (Cantoni et al., 2003). Because of the relatively noninhibitory characteristics of fresh Latin-style cheeses (e.g., pH >6.0), these products allow the proliferation of spoilage organisms and possibly pathogens, if present. Other previously reported spoilage organisms associated with fresh cheese include psychrotolerant gram-negative rods (e.g., *Pseudomonas* spp.), lactic acid bacteria, yeasts, and molds (Bibek, 1996; Mendoza-Yepes et al., 1999). These types of spoilage organisms most frequently contaminate products after processing.

A recent issue of blue pigment-producing *P. fluorescens* on the surface of Mozzarella cheese in Italy caused worldwide news coverage and the spoilage of 70,000 pieces of cheese (Santella and Schmitt-Roschmann,

Table 3. Characteristics of selected isolates from Queso Fresco and environmental swabs and comparison isolates from an unrelated study

FSL no. ¹	Isolate ID ²	Isolate origin (sampling) ³	Color development on PDA ⁴	Fluorescence under UV ⁵	Cheese inoculation ⁶	Isolate similarity for selected tests ⁷	
						PFGE	Ribotype
W5-203	<i>Pseudomonas fluorescens</i>	PT-1 cheese curd (1)	Blue	Yes	Blue	1	1
W5-206	<i>P. fluorescens</i>	PT-1 cheese curd (1)	Blue	Yes	Blue	1	1
W5-207	<i>P. fluorescens</i>	PT-1 cheese curd (1)	Blue	Yes	Blue	1	1
W5-208	<i>P. fluorescens</i>	PT-1 cheese curd (1)	Blue	Yes	Blue	1	1
W5-287	<i>P. fluorescens</i>	PT-2 cheese curd (1)	Blue	Yes	Blue	1	1
W5-296	<i>P. fluorescens</i>	PT-2 cheese curd (1)	Blue	Yes	Blue	2	1
W5-319	<i>P. fluorescens</i>	CV-2 agitator drain tube swab (3)	No color	No	No color	3	1
W5-325	<i>P. fluorescens</i>	CV-1 agitator track swab (3)	Blue	Yes	Blue	1	1
W5-326	<i>P. fluorescens</i>	CV-1 agitator track swab (3)	Blue	Yes	Blue	1	1
W5-327	<i>P. fluorescens</i>	CV-1 agitator track swab (3)	No color	No	No color	3	1
R5-199*	<i>P. fluorescens</i>	Dairy plant isolate	No color	No	No color	NA	1
R5-202*	<i>P. fluorescens</i>	Dairy plant isolate	No color	No	No color	NA	1

¹Isolate ID number always includes prefix FSL (Food Safety Laboratory; e.g., FSL W5-203); all isolates tested were gram-negative, catalase-positive and oxidase-positive rods; comparison isolates from an unrelated study are indicated by asterisk.

²Identification based on 16S rDNA sequencing. API (BioMerieux Vitek Inc., Hazelwood, MO) also identified all isolates as *Pseudomonas fluorescens*, whereas Biolog (Biolog Inc., Hayward, CA) identified all isolates as *Pseudomonas fluorescens* with the exception of R5-199 and R5-202, which were identified as *Pseudomonas tolaasii* and *Pseudomonas synxantha*, respectively.

³PT = press table; CV = cheese vat; samplings 1, 2, and 3 indicate different dates on which samples were collected.

⁴Isolates were streaked onto potato dextrose agar (PDA), incubated, and then observed for color development.

⁵Isolates were streaked onto PDA and then observed for fluorescence under UV light.

⁶Isolates were inoculated onto fresh low-acid cheese, which was incubated and then observed for color development.

⁷Results from selected tests were compared for similarity between isolates; isolates with identical characteristics are designated by same number. PFGE = pulsed field gel electrophoresis; NA = isolates not tested.

Figure 1. Photographs of fresh, low-acid cheese inoculated with *Pseudomonas fluorescens* biovar IV, followed by incubation at 6°C for 48 h, exhibiting (A) blue surface pigmentation and (B) fluorescence under UV light.

2010). The source of the contamination was traced back to the water supply used for brining the cheese. This type of high-profile outbreak of spoilage organisms further emphasizes the need for subtyping tools that can track sources of contamination.

Molecular Subtyping Tools Are Useful in Tracking Potential Sources of Contamination

The presence of 2 pigment-producing *P. fluorescens* isolates from environmental swabs that had the same PFGE pattern as the contaminated cheese curd isolates gave some insight into the potential source of the cheese contamination. These 2 isolates were collected from environmental swabs taken from the agitator

track above CV-1, which may be the source of the contamination. Bacterial tracking of *P. fluorescens* in dairy processing environments has been studied previously (Ralyea et al., 1998), using automated ribotyping as a method to track postpasteurization contamination. Ribotyping has also been used to understand contamination patterns and track pathogenic bacteria such as *Listeria monocytogenes* in Latin-style cheese processing facilities (Kabuki et al., 2004). In this study, ribotyping was found to lack the discriminatory power needed to distinguish between pigment-producing isolates and non-pigment-producing isolates, whereas PFGE was capable of distinguishing between them. Pulsed field gel electrophoresis has previously been shown to have superior discriminatory power over other methods of molecular subtyping (Wiedmann, 2002; Meays et al., 2004; Fugett et al., 2007).

Discriminatory power is just one challenge to source tracking. Often the more difficult question to answer is the directionality of the contamination. Simply knowing that the same organism exists in the environment and the product does not imply that the environment contaminated the product; in fact, it may indicate that the product contaminated the environment or that an undetected source has contaminated both the product and the environment. In this case, the plant had worked extensively with their cleaning chemical supply company on microbiological testing of swabs collected from product contact surfaces and evaluating cleaning procedures of related equipment with no specific leads to potential sources; the agitator system was not considered, however. It was observed that the cheese vat agitator track had residual soils and grease and that the agitator track drains located above cheese vats had the potential to drip on the vat product valves. It was also observed that plant personnel would wash and spray one vat while the adjacent vat was unprotected, posing a risk of creating aerosols from the agitator system. Hence, the agitator track represents a likely and plausible source of the contamination issue reported here.

The *P. fluorescens* contamination in this processing facility was ultimately controlled by using a combination of improved plant cleaning and sanitation procedures, the use of starter cultures, and the addition of potassium sorbate (0.15%) during the grinding step of processing. The addition of starter culture as a type of hurdle technique has been recommended to reduce the risk of pathogen growth (Mendoza-Yepes et al., 1999), but has also proved useful in controlling food spoilage organisms. The manufacturer worked with their chemical supplier to improve washing, sanitization, and monitoring procedures (e.g., increased wash temperatures and circuit times). Based on our findings, the agitator system was subjected to an intensive cleaning

and sanitation procedure, and measures to minimize environmental contamination during vat washings (e.g., shielding and vat covers) were also implemented.

ACKNOWLEDGMENTS

This project was supported by the New York State Milk Promotion Advisory Board through the New York State Department of Agriculture and Markets—NYS dairy farmers committed to production of high quality dairy products. The authors thank the staff and students of the Food Safety Laboratory (Cornell University, Ithaca, NY) and the Laboratory of Food Microbiology and Pathogenesis of Foodborne Diseases (Cornell University, Ithaca, NY) for assistance with this study.

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