Development of a Monte Carlo simulation model to predict pasteurized fluid milk spoilage due to post-pasteurization contamination with gram-negative bacteria

S. Lau,¹ A. Trmcic,¹ N. H. Martin,¹ M. Wiedmann,¹ and S. I. Murphy^{2*}

¹Milk Quality Improvement Program, Department of Food Science, Cornell University, Ithaca, NY 14853

²Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY 14853

ABSTRACT

Psychrotolerant gram-negative bacteria introduced as post-pasteurization contamination (PPC) are a major cause of spoilage and reduced shelf life of high-temperature, short-time pasteurized fluid milk. To provide improved tools to (1) predict pasteurized fluid milk shelf life as influenced by PPC and (2) assess the effectiveness of different potential interventions that could reduce spoilage due to PPC, we developed a Monte Carlo simulation model that predicts fluid milk spoilage due to psychrotolerant gram-negative bacteria introduced as PPC. As a first step, 17 gram-negative bacterial isolates frequently associated with fluid milk spoilage were selected and used to generate growth data in skim milk broth at 6°C. The resulting growth parameters, frequency of isolation for the 17 different isolates, and initial concentration of bacteria in milk with PPC, were used to develop a Monte Carlo model to predict bacterial number at different days of shelf life based on storage temperature of milk. This model was then validated with data from d 7 and 10 of shelf life, collected from commercial operations. The validated model predicted that the parameters (1) maximum growth rate and (2) storage temperature had the greatest influence on the percentage of containers exceeding 20,000 cfu/mL standard plate count on d 7 and 10 (i.e., spoiling due to PPC), which indicates that accurate data on maximum growth rate and storage temperature are important for accurate predictions. In addition to allowing for prediction of fluid milk shelf life, the model allows for simulation of “what-if” scenarios, which allowed us to predict the effectiveness of different interventions to reduce overall fluid milk spoilage due to PPC through a set of proof-of-concept scenario (e.g., frequency of PPC in containers reduced from 100% to 10%; limiting distribution temperature to a maximum

of 6°C). Combined with other models, such as previous models on fluid milk spoilage due to psychrotolerant spore-forming bacteria, the data and tools developed here will allow for rational, digitally enabled, fluid milk shelf life prediction and quality enhancement.

Key words: fluid milk, spoilage, post-pasteurization contamination, Monte Carlo simulation

INTRODUCTION

Microbial spoilage is a contributor to food loss and waste globally, and represents a challenge for maintaining the quality and expected shelf life of many foods, including fluid milk (Hoover, 2017; FAO, 2019). In the United States, dairy products are among the top 3 food groups representing the largest share of the total volume of food lost or wasted, with fluid milk responsible for approximately 65% of food wasted in volume attributed to dairy products (Buzby et al., 2014). The value of fluid milk loss in the US is estimated to be \$6.4 billion per year (Buzby et al., 2014). Globally, fluid milk production, processing, and transport is estimated to be responsible for 2.4 kg of CO₂ equivalents (CO₂e) per kilogram of milk (FAO, 2010). Thus, reducing the volume of fluid milk wasted represents an opportunity with potential for economic and environmental impact.

Although the quality of fluid milk and dairy products can be degraded via different mechanisms (e.g., chemical, microbial), microbial growth is the predominant mechanism for pasteurized fluid milk spoilage (Martin et al., 2016). One of the main causes of microbial fluid milk spoilage is post-pasteurization contamination (PPC) with gram-negative bacteria during processing. Existing data show that in the United States approximately 50% of HTST-pasteurized fluid milk spoilage is due to PPC (Alles et al., 2018; Reichler et al., 2018). Although PPC can introduce a variety of different organisms, key organisms of concern for their potential to cause spoilage are those that can grow in refrigerated fluid milk (e.g., around 6°C). Most studies suggest that the majority of fluid milk spoilage is caused by psychro-

Received September 20, 2021.

Accepted November 10, 2021.

*Corresponding author: sim39@cornell.edu

tolerant non-coliform gram-negative bacteria from the genus *Pseudomonas* (Ranieri and Boor, 2009; Martin et al., 2018; Reichler et al., 2018). When these organisms are introduced to fluid milk as a result of PPC, they usually grow to >20,000 cfu/mL over 10 d, resulting in shelf life that is typically at least 5 to 7 d shorter, compared with fluid milk that is only contaminated by psychrotolerant gram-positive spore-formers (Ranieri and Boor, 2009). Identification of strategies to reduce PPC has potential to reduce the overall amount of fluid milk spoilage and extend shelf life, which would hence reduce fluid milk waste.

Both identification of PPC and implementation of PPC control strategies can be challenging. Key challenges with identification of PPC include, but are not limited to the following: (1) PPC can often occur at low levels (e.g., <10 cfu per container), requiring tedious microbiological methods for identification; (2) the nature of contamination is frequently sporadic; and (3) a large number of possible root causes for PPC exist, ranging from improperly designed or implemented cleaning and sanitation approaches to procedures that facilitate cross-contamination (Sunga et al., 1970; Schröder, 1984; Gruetzmacher and Bradley, 1999; Reichler et al., 2018). Interventions to reduce PPC in a plant can include enhancing good manufacturing practices and cleaning sanitation procedures, improving preventative maintenance, as well as improving sanitary equipment and plant design (Reichler et al., 2020; Murphy et al., 2021). Another hurdle to reducing PPC is the difficulty of assessing the expected effects of interventions on fluid milk shelf life, thus hampering the ability of companies to assess the potential return on investment that could be achieved with different interventions.

One solution to support improved decision-making regarding selection and implementation of PPC interventions is to use modeling approaches. To that end, we report the development and validation of a Monte Carlo simulation model of pasteurized fluid milk spoilage due to PPC to estimate the concentration of gram-negative bacteria throughout shelf life and predict the effects of different intervention strategies.

MATERIALS AND METHODS

Identification and Selection of Bacterial Representatives of PPC

Data reported in a previous comprehensive study of pasteurized fluid milk spoilage in 10 processing plants across the northeastern United States (Reichler et al., 2018) were used to select representative bacterial genera, species, and strains that are commonly associated with fluid milk spoilage due to PPC, for determina-

tion of growth parameters needed for the development of the fluid milk model reported here. In the Reichler et al. (2018) study, a total of 459 gram-negative isolates obtained from fluid milk samples with PPC were characterized by 16S rDNA sequencing and assigned a sequence type (ST) based on an approximately 552-nucleotide region (Reichler et al., 2018). Data from 16S rDNA were also used to assign isolates to genus and species. In this study, using the Reichler et al. (2018) data set, *Pseudomonas* groups were then assigned to 1 of 8 phylogenomic groups based on the species (i.e., groups of related *Pseudomonas* species, such as the *Pseudomonas fluorescens* group), adapted from the group assignment method used by Garrido-Sanz et al. (2016). Specifically, isolates from the Reichler et al. (2018) data set were assigned to the previously defined groups from the Garrido-Sanz et al. (2016) study based on species; we then divided the largest group (*P. fluorescens* group) into clades. Data on the frequency of different ST among the characterized 459 isolates of gram-negative bacteria (Table 1) were used to select 17 isolates for growth experiments.

Analysis of the Reichler et al. (2018) data set revealed that the majority of isolates linked to PPC were identified as *Pseudomonas* spp. (324 out of 459 isolates). Fifteen *Pseudomonas* ST were isolated ≥ 5 times and represented the most frequent *Pseudomonas* ST identified in this study. These 15 ST represented 5 of the 9 proposed *Pseudomonas* groups (e.g., *P. fluorescens* group, *Pseudomonas fragi* group), but also included 1 ST (ST 24) that could not be unambiguously classified to species and may represent 1 of 2 *Pseudomonas* groups (*Pseudomonas mandelii* or *Pseudomonas corrugata*). The ST classified into the *P. fluorescens* group represented 7/15 *Pseudomonas* ST isolated ≥ 5 times and 4 of the 6 major 16S clades within the *P. fluorescens* group. To provide representation of the *Pseudomonas* most commonly associated with PPC, we selected (1) the ST most commonly isolated in each of the 4 *P. fluorescens* clades (e.g., *P. fluorescens* clade 5; Table 1) as well as (2) the ST found most frequently among each of the 4 other *Pseudomonas* groups that were represented among this data set; the specific isolate representing each ST was randomly selected (using a random number generator) for inclusion in the growth experiments. Although this process yielded 8 different *Pseudomonas* isolates (4 from the *P. fluorescens* group and 4 from other *Pseudomonas* groups, including *P. fragi*, *Pseudomonas chloraphis*, *Pseudomonas putida*, and *P. mandelii*) that were used for growth curve experiments, a *P. chloraphis* isolate initially selected was removed from the final data set due to a contamination event.

In addition to *Pseudomonas*, the isolates reported by Reichler et al. (2018) as linked to PPC also included

Table 1. Numbers and prevalence of gram-negative 16S sequence types (ST) obtained from pasteurized milk collected from 10 processing plants over 3.5 years based on data reported by Reichler et al. (2018)

Genus	Species ¹	ST	Total number of isolates across 459 isolates	Frequency (%) ²	Selected for growth experiments	<i>Pseudomonas</i> group for ST isolated ≥ 5 times
<i>Pseudomonas</i>	<i>poae</i>	9	41	8.93	X	<i>P. fluorescens</i>
<i>Pseudomonas</i>	<i>fragi</i>	13	41	8.93	X	<i>P. fragi</i>
<i>Pseudomonas</i>	spp.	16	33	7.19		<i>P. fluorescens</i>
<i>Pseudomonas</i>	<i>tolaasii</i>	52	32	6.97	X	<i>P. fluorescens</i>
<i>Pseudomonas</i>	<i>lundensis</i>	11	23	5.01		<i>P. chloraphis</i>
<i>Pseudomonas</i>	<i>orientalis</i>	23	20	4.36	X	<i>P. fluorescens</i>
<i>Pseudomonas</i>	spp.	51	14	3.05	X	<i>P. fluorescens</i>
<i>Pseudomonas</i>	<i>grimontii</i>	100	14	3.05	X	<i>P. fluorescens</i>
<i>Acinetobacter</i>	<i>haemolyticus</i>	2	10	2.18	X	
<i>Stenotrophomonas</i>	<i>rhizophila</i>	41	10	2.18	X	
<i>Pseudomonas</i>	spp.	33	8	1.74		<i>P. fluorescens</i>
<i>Acinetobacter</i>	<i>guillouiae</i>	1	7	1.53		
<i>Lelliottia</i>	<i>amnigena</i>	17	7	1.53	X	
<i>Pseudomonas</i>	<i>taiwanensis_fulva_plecoglossicida_sl</i>	6	6	1.31	X	<i>P. putida</i>
<i>Hafnia</i>	<i>parabvei</i>	36	6	1.31	X	
<i>Rahnella</i>	<i>aquatilis</i>	56	6	1.31	X	
<i>Pseudomonas</i>	spp.	85	6	1.31		<i>P. fluorescens</i>
<i>Pseudomonas</i>	<i>brassicacearum_rmigulae_sl</i>	24	5	1.09	X	
<i>Pseudomonas</i>	spp.	67	5	1.09		
<i>Pseudomonas</i>	spp.	78	5	1.09		
<i>Pseudomonas</i>	spp.	82	5	1.09		
<i>Pseudomonas</i>	spp.	10	4	0.87		
<i>Raoultella/Kluyvera</i>	spp.	71	4	0.87		
<i>Pantoea</i>	<i>anthophila</i>	75	4	0.87	X	
<i>Pseudomonas</i>	spp.	83	4	0.87		
<i>Limnohabitans</i>	<i>cf_planktonicus</i>	3	3	0.65		
<i>Comamonas</i>	<i>cf_korensis</i>	4	3	0.65		
<i>Pseudomonas</i>	spp.	14	3	0.65		
<i>Psychrobacter</i>	spp.	30	3	0.65		
<i>Comamonas</i>	<i>cf_thiooxydans</i>	54	3	0.65		
<i>Obesumbacterium</i>	<i>proteus</i>	81	3	0.65	X	
<i>Pseudomonas</i>	spp.	128	3	0.65		
<i>Pseudomonas</i>	spp.	15	2	0.44		
<i>Raoultella or Citrobacter</i>	spp.	18	2	0.44		
<i>Pseudomonas</i>	spp.	20	2	0.44		
<i>Pseudomonas</i>	spp.	21	2	0.44		
<i>Pseudomonas</i>	spp.	22	2	0.44		
<i>Citrobacter/Raoultella/Kluyvera</i>	spp.	34	2	0.44		
<i>Buttiauxella</i>	spp.	49	2	0.44		
<i>Enterobacter</i>	<i>ludwigii</i>	53	2	0.44		
<i>Pseudomonas</i>	spp.	60	2	0.44		
<i>Serratia</i>	<i>quinivorans</i>	61	2	0.44		
<i>Pseudomonas</i>	spp.	62	2	0.44		
<i>Rahnella</i>	<i>cf_aquatilis</i>	65	2	0.44		
<i>Rahnella</i>	<i>aquatilis</i>	68	2	0.44		
<i>Pseudomonas</i>	spp.	73	2	0.44		
<i>Yersinia</i>	<i>kristensenii_bercovieri_sl</i>	74	2	0.44		
<i>Pseudomonas</i>	spp.	95	2	0.44		
<i>Hafnia</i>	<i>parabvei</i>	96	2	0.44		

Continued

Table 1 (Continued). Numbers and prevalence of gram-negative 16S sequence types (ST) obtained from pasteurized milk collected from 10 processing plants over 3.5 years based on data reported by Reichler et al. (2018)

Genus	Species ¹	ST	Total number of isolates across 459 isolates	Frequency (%) ²	Selected for growth experiments	<i>Pseudomonas</i> group for ST isolated ≥ 5 times
<i>Pseudomonas</i>	spp.	106	2	0.44		
<i>Pseudomonas</i>	spp.	108	2	0.44		
<i>Obesumbacterium</i>	<i>proteus</i>	110	2	0.44		
<i>Pseudomonas</i>	spp.	116	2	0.44		
<i>Pseudomonas</i>	spp.	117	2	0.44		
<i>Pseudomonas</i>	spp.	127	2	0.44		
<i>Pseudomonas</i>	spp.	132	2	0.44		
<i>Pseudomonas</i>	spp.	146	2	0.44		
<i>Citrobacter</i>	<i>farneri</i>	5	1	0.22		
<i>Aeromonas</i>	<i>jandaei_media_hydrophila_sl</i>	7	1	0.22		
<i>Pseudomonas</i>	spp.	8	1	0.22		
<i>Pseudomonas</i>	spp.	12	1	0.22		
<i>Janthinobacterium</i>	<i>lividum</i>	19	1	0.22		
<i>Pseudomonas</i>	spp.	35	1	0.22		
<i>Hafnia</i>	<i>parabvei</i>	37	1	0.22		
<i>Hafnia</i>	<i>parabvei</i>	38	1	0.22		
<i>Pseudomonas</i>	spp.	39	1	0.22		
<i>Hafnia</i>	<i>parabvei</i>	40	1	0.22		
<i>Hafnia</i>	<i>parabvei</i>	42	1	0.22		
<i>Janthinobacterium</i>	<i>lividum</i>	43	1	0.22		
<i>Citrobacter</i>	<i>gillenii</i>	46	1	0.22		
<i>Shewanella</i>	<i>hafniensis</i>	47	1	0.22		
<i>Buttiauxella</i>	<i>brennerae</i>	48	1	0.22	X	
<i>Pseudomonas</i>	spp.	50	1	0.22		
<i>Pseudomonas</i>	spp.	57	1	0.22		
<i>Pseudomonas</i>	spp.	59	1	0.22		
<i>Raoultella</i>	<i>ornithinolytica</i>	63	1	0.22		
<i>Pseudomonas</i>	spp.	64	1	0.22		
<i>Cedecea</i>	<i>davisae</i>	66	1	0.22		
<i>Acinetobacter</i>	<i>haemolyticus</i>	70	1	0.22		
<i>Massilia</i>	<i>aurea</i>	72	1	0.22		
<i>Janthinobacterium</i>	<i>lividum</i>	76	1	0.22		
<i>Pseudomonas</i>	spp.	77	1	0.22		
<i>Hafnia</i>	<i>parabvei</i>	79	1	0.22		
<i>Pseudomonas</i>	spp.	80	1	0.22		
<i>Obesumbacterium</i>	<i>proteus</i>	84	1	0.22		
<i>Klebsiella</i>	<i>pneumoniae</i>	86	1	0.22		
<i>Pseudomonas</i>	spp.	87	1	0.22		
<i>Pseudomonas</i>	spp.	88	1	0.22		
<i>Pantoea</i>	<i>anthrophila</i>	89	1	0.22		
<i>Pseudomonas</i>	spp.	90	1	0.22		
<i>Hafnia</i>	<i>cf_parabvei</i>	91	1	0.22		
<i>Serratia</i>	<i>proteamaculans</i>	92	1	0.22	X	
<i>Buttiauxella</i>	<i>brennerae</i>	93	1	0.22		
<i>Citrobacter</i>	<i>gillenii</i>	94	1	0.22		
<i>Pseudomonas</i>	spp.	97	1	0.22		
<i>Obesumbacterium</i>	<i>cf_proteus</i>	98	1	0.22		
<i>Hafnia</i>	<i>cf_parabvei</i>	99	1	0.22		
<i>Janthinobacterium</i>	<i>lividum</i>	101	1	0.22		

Continued

Table 1 (Continued). Numbers and prevalence of gram-negative 16S sequence types (ST) obtained from pasteurized milk collected from 10 processing plants over 3.5 years based on data reported by Reichler et al. (2018)

Genus	Species ¹	ST	Total number of isolates across 459 isolates	Frequency (%) ²	Selected for growth experiments	<i>Pseudomonas</i> group for ST isolated ≥ 5 times
<i>Janthinobacterium</i>	<i>lividum</i>	102	1	0.22	X	
<i>Brevundimonas</i>	<i>vesicularis</i> (T); <i>LMG_2350</i>	105	1	0.22		
<i>Pseudomonas</i>	spp.	107	1	0.22		
<i>Chryseobacterium</i>	<i>shigense_luteum_sl</i>	109	1	0.22		
<i>Pseudomonas</i>	spp.	111	1	0.22		
<i>Pseudomonas</i>	spp.	112	1	0.22		
<i>Citrobacter</i>	<i>murlinae_braakii_sl</i>	113	1	0.22		
<i>Yersinia</i>	<i>kristensenii</i>	114	1	0.22		
<i>Pseudomonas</i>	spp.	115	1	0.22		
<i>Pseudomonas</i>	spp.	119	1	0.22		
<i>Serratia</i>	<i>grimesii</i>	120	1	0.22		
<i>Serratia</i>	<i>sp_glossinae_marcescens</i>	122	1	0.22		
<i>Sphingobacterium</i>	<i>kitahiroshumense</i>	125	1	0.22		
<i>Pseudomonas</i>	spp.	129	1	0.22		
<i>Pseudomonas</i>	spp.	131	1	0.22		
<i>Obesumbacterium/Hafnia</i>	<i>proteus_paralvei_sl</i>	133	1	0.22		
<i>Obesumbacterium</i>	<i>proteus</i>	134	1	0.22		
<i>Obesumbacterium/Hafnia</i>	<i>proteus_paralvei_sl</i>	135	1	0.22		
<i>Hafnia</i>	<i>alvei</i>	141	1	0.22		
<i>Sphingobacterium</i>	<i>kitahiroshimense</i>	142	1	0.22		
<i>Rahnella</i>	<i>aquatilis</i>	143	1	0.22		
<i>Butiaurella</i>	<i>izardii</i>	144	1	0.22		
<i>Lecrercia</i>	<i>adecarboxylata</i>	145	1	0.22		
<i>Serratia</i>	<i>quinivorans</i>	147	1	0.22		
<i>Pseudomonas</i>	spp.	148	1	0.22		
<i>Janthinobacterium</i>	<i>sp_agaricidamnosum_lividum</i>	149	1	0.22		
<i>Janthinobacterium</i>	<i>lividum</i>	150	1	0.22		

¹sl = *sensu lato* ("in the broad sense").²Due to significant digits, the total frequency adds up to above 100%.

the genera *Buttiauxella*, *Hafnia*, *Lelliottia*, *Obesumbacterium*, *Pantoea*, *Rahnella*, *Serratia*, *Janthinobacterium*, *Acinetobacter*, *Comamonas*, and *Stenotrophomonas*. For 10 of these 11 genera of gram-negative bacteria, 1 isolate representing the most common ST was randomly selected for inclusion in the growth experiments (a *Comamonas* isolate was not included, due to a laboratory error); if multiple ST included the same number of isolates (e.g., each of the 5 *Janthinobacterium* isolates represented a different ST), 1 ST was randomly selected. *Janthinobacterium* spp. isolate FSL R10-0933 (ST 76), which was initially selected, did not grow in brain heart infusion (BHI) broth incubated at 32°C and was replaced with another *Janthinobacterium* spp. isolate, FSL R10-1587 (ST 102). Overall, this approach yielded 17 isolates (7 *Pseudomonas*, 7 *Enterobacteriaceae*, and 3 other genera of non-*Enterobacteriaceae* gram-negative bacteria) that were used for growth experiments (Table 2). The FSL numbers of strains refer to the Food Science Laboratory at Cornell University. Additional isolate information can be found via the Food Microbe Tracker Database (www.foodmicrobetracker.com; Van-gay et al., 2013).

Determination of Cold-Growth Characteristics at 6°C

To determine cold-growth characteristics in skim milk broth (SMB; Becton, Dickinson and Co.), all selected isolates were streaked from frozen culture stocks onto BHI agar (Becton, Dickinson and Co.). Following 24-h incubation at 32°C, a single colony was selected from the BHI plate and used to inoculate 5 mL of BHI broth, which was subsequently incubated overnight (approximately 12–18 h) at 32°C. Bacterial numbers in the overnight BHI cultures were enumerated by plating appropriate dilutions on BHI plates, which were subsequently incubated at 32°C for 24 h; the undiluted overnight cultures were stored during these 24 h at 4°C before being used for inoculation of SMB. The enumeration results were used to determine the volume and dilution necessary to inoculate SMB at a starting concentration of approximately 10^3 cfu/mL. The inoculated SMB was incubated at 6°C, and bacterial numbers were enumerated (with 2 technical replicates) every 4 h during the first 12 h to capture the lag phase, every 12 h during the next 60 h, and every 24 h until 2 time points were taken during the stationary phase so that we could fully capture the exponential phase. The cold-growth characteristics for each isolate were determined based on at least 3 separate biological replicates of the experiment. Isolate cold-growth data were managed in Excel (version 2108, Microsoft Corp.) and were joined and cleaned in R version 3.6.2 (R Core Team, 2019).

Table 2. Genus and species identification, 16S sequence type (ST), and growth parameters for the 17 isolates from pasteurized fluid milk¹

Genus	Species ²	FSL ID	ST	Lag ₃ (h)	$\mu_{\max}$ (ln cfu/h)	$\mu_{\max_adj}$ (ln cfu/h)	$N_{\max}$ (log ₁₀ cfu/mL)	Best-fitting growth model
<i>Pseudomonas</i>	<i>fragi</i>	R10-0056	13	25.2	0.100	0.069	9.07	Gompertz
	<i>poae</i>	R10-0084	9	<4	0.107	0.073	8.39	Gompertz
	<i>tolaasi</i>	R10-0553	52	11.6	0.089	0.061	8.38	Gompertz
	<i>orientalis</i>	R10-0908	23	25.5	0.125	0.086	8.08	Gompertz
	<i>grinonii</i>	R10-1432	100	<4	0.141	0.096	8.04	Gompertz
	<i>taiwanensis_fulva_ptecoglossicida_sl</i>	R10-0990	6	20.9	0.098	0.067	7.81	Gompertz
	<i>brassicacearum_migulac_sl</i>	R10-0151	24	7.4	0.086	0.059	8.32	Gompertz
<i>Buttiauxella</i>	<i>brennerae</i>	R10-0531	48	34.9	0.081	0.056	8.51	Baranyi
	<i>parvlei</i>	R10-3286	36	<4	0.114	0.078	9.04	Gompertz
	<i>amniigena</i>	R10-1099	17	26.3	0.079	0.054	9.08	Gompertz
	<i>proteus</i>	R10-1113	81	28.4	0.078	0.053	9.20	Gompertz
	<i>anthophila</i>	R10-0941	75	<4	0.085	0.058	8.57	Gompertz
	<i>aquatilis</i>	R10-0701	56	11.2	0.122	0.083	8.24	Baranyi
	<i>proteamaculans</i>	R10-1344	92	12.3	0.133	0.091	8.89	Gompertz
<i>Janthinobacterium</i> ⁴	<i>lividum</i>	R10-1587	102	<4	0.058	0.040	7.82	Gompertz
	<i>Acinetobacter</i>	R10-2381	2	20.4	0.094	0.064	7.35	Gompertz
<i>Stenotrophomonas</i>	<i>rhizophila</i>	R10-1181	41	7.4	0.075	0.051	8.17	Baranyi

¹FSL ID = Food Science Laboratory (Cornell University) identification; $\mu_{\max}$ = maximum growth rate by ST; $\mu_{\max_adj}$ = adjusted maximum growth rate by ST; $N_{\max}$ = maximum microbial population by ST.

²sl = *sensu lato* ("in the broad sense").

³<4; during first 12 h of the growth experiments, bacterial cultures were enumerated every 4 h.

⁴The initial *Janthinobacterium* isolate chosen for growth experiments was isolate FSL R10-0933; this isolate did not grow in liquid brain heart infusion medium, and *Janthinobacterium lividum* isolate FSL R10-1587 was chosen as a replacement isolate.

Determining Cold-Growth Parameters

The *nlsmicrobio* package version 0.0-1 (Baty and Delignette-Muller, 2017) in R version 3.6.2 (R Core Team, 2019) was used to fit the data for the 3 biological replicates to the Buchanan (Buchanan et al., 1997), Baranyi (Baranyi and Roberts, 1994), and Gompertz (Zwietering et al., 1991) growth models (Table 2). The Bayesian information criterion values calculated with the *AICcmodavg* package version 2.3-1 (Mazerolle 2020) in R version 3.6.2 were used to select the growth model with the best fit. Initially, for each ST, the bacterial enumeration data for each time point were averaged across biological replicates. The averaged data were plotted to estimate the starting values of the growth parameters; these starting value estimates allowed for convergence to be achieved and allowed us to use the iterative estimation procedure in the *nlsmicrobio* package version 0.0-1 to best fit the data (Baty et al., 2015). The averaged data were then fitted with the growth models; this approach allowed us to identify a single growth model that best fit the data; the best-fit model was selected based on Bayesian information criterion values. Fitting the averaged values also allowed for identification of outliers, using all the growth data over time. To identify potential outliers of the bacterial enumeration data, residuals of individual data points with respect to the best-fit model were retrieved; data points were considered outliers if their residuals were identified as outliers among all residuals, based on the interquartile range method (Vinutha et al., 2018). After removal of the outliers, the remaining bacterial enumeration data were fitted with the best-fit growth model again, and the growth parameters were determined for the respective ST, including (1) lag phase (h), (2) maximum growth rate ($\mu_{\max}$, ln cfu/h), and (3) maximum cell density ($\log_{10}$ cfu/mL; Table 2).

Predictive Model Development

Model Assumptions. For the model developed here, PPC organisms were assumed to be the only cause of spoilage in each simulated half-gallon of milk (1,900 mL). Furthermore, each simulated half-gallon of milk was assigned an initial concentration, storage temperature, and ST. Each half-gallon container of milk was assumed to be stored at a constant temperature during shelf life. The baseline model comprises 10,000 iterations, and each iteration is considered to represent one lot. Here, a lot is defined as 10 fluid milk containers coming from the same processing facility and the same processing day, modeled across 14 d of shelf life; as a result, a total of 100,000 containers are simulated. For the baseline model, it was assumed that the frequency

of PPC for the 10 half-gallon containers in all simulated lots was 100% (i.e., all simulated containers had PPC); however, if the assigned initial concentration was less than 1 cfu per half-gallon, it was assumed that PPC did not occur in that container.

The effects of lower frequencies of PPC using this model can be assessed by assuming that the half-gallon containers modeled here represent only a certain portion of a given lot; for example, if the processing plant expects that only 50% of a lot are to be affected by PPC, then the model developed here would represent 50% of the processing plant's lot. The other 50% of the lot would be expected to spoil due to psychrotolerant spore-forming bacteria, and spoilage patterns could be assessed with a previously published "spore-former model" (Buehler et al., 2018), or, in the case of fewer than 1 psychrotolerant spore per container, not spoil during the storage period.

When conducting our analysis, we used 20,000 cfu/mL SPC as the microbial threshold for defining spoilage in this study. This value is the upper limit of the concentration a container can reach before it is deemed unacceptable from a quality standpoint, or what we refer to as spoiled fluid milk. We appreciate that this is a conservative threshold (which ensures compliance with the US Pasteurized Milk Ordinance limits for pasteurized milk; FDA, 2019); an alternative threshold can be easily used by this model if so desired.

Model Parameters. Six parameters were included in the model developed here (Table 3), including (1) storage temperature (**T**), (2) initial concentration of gram-negative bacteria in pasteurized milk (N_0), (3) frequency of different 16S rDNA ST of gram-negative bacteria as PPC (**F_{ST}**), (4) adjusted maximum growth rate by ST ($\mu_{\max_adj}$), (5) lag phase by ST (t_{lag}), and (6) maximum microbial population by ST (N_{max}).

For parameter (1), storage temperature (**T**), a temperature distribution following a modified Laplace distribution was used (Table 3), based on a previous study that analyzed home refrigeration temperatures from a 2005 national survey in the US collected by Research Triangle Institute International (Pouillot et al., 2010). The minimum and maximum temperatures that could be drawn from the distribution were set at -1°C and 15°C , respectively, to prevent unrealistically low or high storage temperatures.

No primary data were available to estimate parameter (2), the initial concentration of gram-negative bacteria (N_0). Values for this parameter were thus derived from total gram-negative count data obtained during shelf life of commercially produced HTST milk collected by Cornell University's Milk Quality Improvement Program Voluntary Shelf Life (**VSL**) Program between January 2015 and June 2018 (N. Martin, unpublished

data). Briefly, total gram-negative counts were determined by plating on crystal violet tetrazolium agar (CVTA), followed by incubation at 21°C for 48 h, and then enumeration. The VSL program has been previously described (Martin et al., 2012; Murphy et al., 2021). The criteria for microbial count data used as N_0 represent only samples that were shown to spoil due to gram-negative bacteria; samples had to have enumeration of gram-negative organisms on CVTA plates. Samples that were below the detection limit, samples that had only 1 data point across shelf life, and samples that showed low levels of bacterial growth on CVTA (<1,000 cfu/mL) were not included. Of the remaining samples, we excluded those that showed low levels of bacterial growth on SPC (<20,000 cfu/mL), which would be indicative of spoilage due to spore-formers, including *Paenibacillus*, which may show growth on CVTA (N. Martin, unpublished data). Microbial count data during shelf life of the 83 samples deemed to have spoiled due to growth by gram-negative bacteria were used to estimate N_0 , assuming linear growth over shelf life; a least squares regression was used to determine initial microbial concentration at d 1, which is one day after processing. With this approach, we determined a log normal distribution for this input parameter (mean = $0.38 \log_{10}$ cfu/mL, SD = $1.11 \log_{10}$ cfu/mL; Figure 1). The maximum initial concentration for each simulated half-gallon was set at $3 \log_{10}$ cfu/mL to avoid simulations with unrealistically high N_0 .

Parameter (3), the frequency of spoilage due to different ST (F_{ST}), represents the expected frequency (percentage of half-gallon containers) with which different ST occur as PPC. The frequency of an individual ST in this study was assumed to be the frequency with which that ST was isolated from fluid milk samples; for example, ST 15, which represented 0.436% of isolates characterized by Reichler et al. (2018), was assumed to be responsible for PPC of 0.436% of modeled half-gallon containers.

Growth characteristics [i.e., $\mu_{\max_adj}$, t_{lag} , and $N_{\max}$; parameters (4), (5), and (6)] for the 17 different ST were obtained from the experimental data and subsequent growth modeling detailed earlier; values of each parameter for a given ST are shown in Table 2. Ratkowsky's square root model $\left[\sqrt{r} = b(T - T_0)\right]$ with a T_0 value of -4.15°C was used to adjust t_{lag} and $\mu_{\max}$ for different temperatures (Ratkowsky et al., 1982). This approach yielded growth parameters for 17 of 124 ST that could cause PPC in the model developed here. Growth parameters were assigned to the other 107 ST using an approach similar to that previously reported by Buehler et al. (2018). Briefly, 16S rDNA sequence data for each of the ST without experimentally ob-

tained growth parameters ($ST_{\text{no exp growth}}$) were used to determine the most closely related ST with experimental growth data ($ST_{\text{exp growth}}$). For this, Basic Local Alignment Search (BLAST) was used to first determine the $ST_{\text{exp growth}}$ that showed the highest percentage of nucleotide similarity to each $ST_{\text{no exp growth}}$; a partial 16S rDNA maximum-likelihood phylogenetic tree of all 124 ST (Supplemental Figure S1, <https://doi.org/10.7298/yth9-zq08>; Lau et al., 2021), built based on data from Reichler et al. (2018), was used to confirm assignment of the most closely related $ST_{\text{exp growth}}$ in a situation where $ST_{\text{no exp growth}}$ shows equal percent similarity to 2 or 3 $ST_{\text{exp growth}}$. In cases where the maximum-likelihood tree would not allow for identification of the most closely related $ST_{\text{exp growth}}$, the growth parameters of the $ST_{\text{exp growth}}$ most closely related to an $ST_{\text{no exp growth}}$ were averaged, and the growth model with the lowest Bayesian information criterion value was selected as the best-fitting model.

Model Simulations. The simulation model was programmed in R version 3.6.2 (R Core Team, 2019), predicting the concentration of PPC organisms in milk containers across 14 d of shelf life. The model was simulated for 10,000 iterations, with 10 half-gallon containers simulated per each iteration. Conceptually, this represents 10,000 unique lots and 10 half-gallon containers of milk per lot, for a total of 100,000 simulated half-gallon containers of milk. Raw data and the R code can be found on GitHub: https://github.com/FSL-MQIP/MC-2020/tree/master/Sam_FFAR_PPCmodel.

Model Validation. The model was validated with d 7 and d 10 shelf life data for milk collected by Cornell's VSL program from commercial operations between July 2018 and May 2019 (N. Martin, unpublished data); this allowed us to validate the model with data independent from those used to estimate input parameter N_0 (i.e., initial concentration of gram-negative bacteria). A total of 127 samples from this data set were used to validate the model for d 7, and a total of 145 samples from this data set were used to validate the model for d 10. These samples were identified as having spoiled due to growth by gram-negative bacteria (using the same criteria used to identify samples for estimation of N_0) and were thus deemed to represent PPC spoilage. For validation, the model was set to use a constant storage temperature of 6°C (rather than the modified Laplace distribution in Table 3) to reflect the storage conditions of the VSL samples used for validation. The Kolmogorov-Smirnov test, implemented using the dgof package version 1.2 (Arnold and Emerson, 2013), was used to compare the distribution of simulated counts and observed counts for 7 d and 10 d in R version 3.6.2 (R Core Team, 2019).

Table 3. Parameters used in the Monte Carlo simulation of growth of cold-tolerant gram-negative bacteria in pasteurized milk

Parameter	Symbol	Variable	Unit	Distribution, value, or formula	Source
1	T	Storage temperature	°C	Laplace distribution (4.06°C, 2.31°C) with minimum and maximum temperature of -1°C and 15°C	Pouillot et al., 2010
2	N_0	Initial microbial population	$\log_{10}$ cfu/mL	Log normal distribution (0.38, 1.11)	Calculated
3	F_{ST}	16S sequence-type frequency	%	See Table 1	Reichler et al., 2018
4	μ_{max_adj}	Adjusted maximum growth rate	ln cfu/h	$\mu_{max_adj} = \mu_{max} \times 0.684$ Buchanan: Lag phase For $t \leq t_{lag}$ $N_t = N_0$ Exponential growth phase For $t_{lag} < t < t_{max}$ $N_t = N_0 + \mu(t - t_{lag})$ Stationary phase For $t \geq t_{max}$ $N_t = N_{max}$ Gompertz: $N_t = N_0 + (N_{max} - N_0) \left[e^{-e^{\mu(t_{lag}-t)/(N_{max}-N_0)+1}} \right]$	Based on experimental data reported here; maximum growth rate determined using the Gompertz (Zwietering et al., 1991), Baranyi (Baranyi and Roberts 1994), or Buchanan model (Buchanan et al., 1997)
5	t_{lag}	Lag	h	Baranyi: $N_t = N_0 + \mu A(t) - \ln \left[1 + \frac{e^{\mu A(t)} - 1}{e^{(N_{max}-N_0)}} \right]$ $A(t) = t + \frac{1}{\mu} \ln \left[e^{-\mu t} + e^{-\mu t_{lag}} - e^{-\mu(t+t_{lag})} \right]$ See μ_{max_adj}	Based on experimental data reported here; lag phase determined using the Gompertz (Zwietering et al., 1991), Baranyi (Baranyi and Roberts 1994), or Buchanan model (Buchanan et al., 1997)
6	N_{max}	Maximum microbial population	$\log_{10}$ cfu/mL	See μ_{max_adj}	Based on experimental data reported here; maximum microbial population determined using the Gompertz (Zwietering et al., 1991), Baranyi (Baranyi and Roberts 1994), or Buchanan model (Buchanan et al., 1997)

Figure 1. Initial concentrations of gram-negative bacteria in pasteurized milk extrapolated from 83 samples collected from January 2015 to June 2018. A least squares regression equation was used to extrapolate the d-1 initial concentration. Sample data below the detection limit were treated as 25% of the limit value (e.g., a detection limit of 20 cfu/mL would be counted as 5 cfu/mL). A log normal distribution (black line overlay) was fitted to the extrapolated d-1 values with a mean of 0.38 $\log_{10}$ cfu/mL and SD of 1.11 $\log_{10}$ cfu/mL.

Sensitivity Analysis. Five model parameters, (1) storage temperature (T), (2) initial microbial concentration (N_0), (3) frequency (F_{ST}), (4) adjusted maximum growth rate ($\mu_{\max_adj}$), and (5) lag (t_{lag}), were included in the sensitivity analysis to determine the relative effects of each parameter on the percentage of containers predicted to spoil due to PPC (i.e., >20,000 cfu/mL SPC and presence of PPC) on d 7 and d 10 of shelf life; d 7 and d 10 were selected for analyses because, if there was spoilage due to PPC, this would be captured through bacterial counts on these 2 d, and data is available for d 7 and 10 with VSL samples for validation. Maximum microbial population ($N_{\max}$) was not assessed for sensitivity analysis because, under the assumption that all milk should have relatively similar concentrations of nutrients, the carrying capacity of bacteria should not change, and thus minimal change in $N_{\max}$ will occur. In one-at-a-time analyses, each respective model parameter (T , N_0 , F_{ST} , $\mu_{\max_adj}$, or t_{lag}) was individually increased or decreased by 20%, 40%, and 60%, while all other model parameters were kept constant. This deterministic approach allowed us to understand the model response to extreme variations of parameter inputs and to compare different parameters with the same level of deviation. For N_0 (the initial microbial concentration) values were on a $\log_{10}$ scale, so the percent reduction was applied to the log value, not the untransformed value (e.g., a 10% reduction translates into a change from 2 $\log_{10}$ to 1.8 $\log_{10}$). For F_{ST} , sensitivity analyses were conducted by removing the ST with the fastest maximum growth rate until ST

that represented 20%, 40%, and 60% of isolates were removed; for example, removal of 15 out of 125 ST that represented the fastest growth rates removed the 20% of isolates that were assigned the fastest growth rates in the original analysis. The same analyses were conducted by removing the ST with the slowest maximum growth rate until ST that represented 20%, 40%, and 60% of isolates were removed; for example, removal of the 51 ST that represented the slowest growth rates removed the 20% of isolates that were assigned the slowest growth rates in the original analysis. The effect of changes to each model parameter was assessed relative to the percentage of half-gallons of milk that were predicted by the baseline model to spoil due to PPC on d 7 and d 10.

What-If Analysis. Four what-if scenarios were used to evaluate the predicted effect of different control measures on the proportion of containers spoiled due to PPC, including (1) reducing the frequency of PPC, (2) reducing the initial concentration of organisms introduced into containers through PPC, (3) eliminating specific ST of concern, including the fastest-growing and most frequent ST, and (4) improving temperature control of fluid milk storage. For scenarios (1) through (3), different levels of change were also assessed (Table 4); henceforth these are referred to as “sub-scenarios.” To determine how many additional days of shelf life different what-if scenarios would provide, we assessed the amount of storage time that was needed to reach a point where 34.32% of half-gallons showed bacteria numbers >20,000 cfu/mL, representing the percentage of half-gallons that reach this threshold by d 7 in the baseline model (Table 4). Day 7 was chosen for assessment of additional days of shelf life because it was one of the 2 d (i.e., d 7 and d 10) validated with data from commercial operations (i.e., VSL data), and validation assessing simulation data and VSL data resulted in closer alignment based on the test statistic for d 7 compared with d 10.

Scenario (1), which models the effects of reducing the frequency of PPC, would represent situations where a processor would implement control strategies with different levels of effectiveness in reducing the frequency with which PPC occurs. This what-if scenario included 2 sub-scenarios, one where the frequency of contaminated containers was reduced from 100% to 50%, which could represent implementation of enhanced cleaning or sanitation strategies (e.g., periodic equipment cleaning), and one where the frequency was reduced from 100% to 10%, which could represent installation of new equipment (e.g., fillers) with improved sanitary design. Scenario (2), which models the effects of reducing the initial concentration of organisms introduced into containers through PPC, would represent situations

where the frequency of contamination stayed similar, but the concentration of PPC organisms introduced would be reduced; for example, through preventative maintenance, which could remove gaskets or fillers that can harbor high concentrations of PPC organisms. Scenario (2) included sub-scenarios where the initial concentration was reduced by 1 or 3 log₁₀, as well as a sub-scenario that reduced initial concentration by 2 log₁₀ for only 10% of containers; this sub-scenario was evaluated to determine the effect of adding an antimicrobial coating to drains. Drains were assumed to be responsible for 10% of PPC, and a previous study showed that an antimicrobial coating can reduce cell density by approximately 2 log₁₀ (Werner et al., 2019). Contamination with aerosolized bacteria from drains can reach milk cartons through the use of high-pressure hoses when cleaning the floors of a processing plant (Kang and Frank, 1990).

Scenario (3), which models the effects of eliminating specific ST, would reflect situations where processors implement targeted strategies to eliminate specific subtypes of PPC organisms (e.g., through application of “Seek and Destroy,” as first described by Butts, 2003); the 2 sub-scenarios included here were (a) elimination of all contamination with ST 100, which had the highest growth rate, and (b) eliminating all contamination with the 2 most frequent ST (ST 13 and 9). For sub-scenarios (a) and (b), we also modeled 2 outcomes of the intervention, including one (“Outcome 1”) where elimination of ST would render any container that would have been contaminated with the eliminated ST or ST as “uncontaminated”; this outcome would be representative of plants with one or few well-defined niches for PPC organisms that can be eliminated through Seek and Destroy. Outcome 2, by contrast, modeled a situation where containers that were contaminated, in the baseline model, with either the ST with the highest growth rate or the 2 most frequent ST, are now contaminated with one of the remaining ST. This outcome would be typical for plants where PPC is caused by many different ST; whereas Seek and Destroy may allow for elimination of specific niches, other contamination sources will remain present and would “fill in” for the ST removed through Seek and Destroy.

Scenario (4) evaluated the effect of improved temperature control on fluid milk spoilage due to PPC; this scenario could include use of temperature sensors to verify effective implementation of this intervention. Here, we truncated the temperature to 6°C, such that the maximum temperature a product can experience is 6°C (simulating stringent temperature control during storage and distribution). Each half-gallon’s assigned temperature was drawn from the previously described modified Laplace distribution; if the temperature drawn

was >6°C, a new temperature would be redrawn from the distribution.

RESULTS

Gram-Negative Bacteria Representatives Commonly Associated with PPC Grow Rapidly at Low Temperatures

All 17 isolates for which cold-growth data were obtained showed >1 log₁₀ growth in SMB incubated at 6°C. Growth modeling yielded (1) estimated lag phases ranging from <4 h to 34.9 h, (2) maximum growth rates ($\mu_{\max}$) ranging from 0.058 to 0.141 ln cfu/h, and (3) maximum cell density ($N_{\max}$) ranging from 7.35 to 9.20 log₁₀ cfu/mL (Table 2). The isolate with the highest $\mu_{\max}$ was *Pseudomonas grimontii*, isolate FSL R10-1432. The lag phase duration and $\mu_{\max}$ of 7 *Pseudomonas* spp. isolates were not significantly different ($P < 0.001$) from the 10 gram-negative bacteria that were not identified as *Pseudomonas* spp. We identified the best-fitting growth model for each of the 17 isolates using the cold-growth data; 14/17 were fitted to the Gompertz model, 3/17 were fitted to the Baranyi model, and 0/17 were fitted to the Buchanan model (Table 2).

Model Output and Validation

Initial validation efforts showed that our model overestimated both the frequency of spoilage due to PPC (i.e., percentage of containers with SPC >20,000 cfu/mL) and mean bacterial counts at d 7 and 10 of shelf life (Supplemental Figure S2, <https://doi.org/10.7298/yth9-zq08>; Lau et al., 2021). To compensate for the observed overestimation, an adjustment factor was computed based on (1) the average $\mu_{\max}$ across all 17 ST_{exp growth} (0.098 ln cfu/h) and (2) the average $\mu_{\max}$ for the VSL samples (0.067 ln cfu/h). We used the obtained ratio of these 2 $\mu_{\max}$ values (0.684) as a multiplier for each of the 17 experimentally obtained $\mu_{\max}$ values, yielding adjusted maximum growth rates ($\mu_{\max_adj}$) for use in the model. The $\mu_{\max_adj}$ ranged from 0.040 to 0.096 ln cfu/h (Table 2). A Kolmogorov-Smirnov test was used to compare the distribution of simulated and observed counts (Figure 2). The Kolmogorov-Smirnov critical value for d 7 was 0.12, and the test statistic was 0.18 ($P < 0.001$). The Kolmogorov-Smirnov critical value for d 10 was 0.11, and the test statistic was 0.24 ($P < 0.0001$). Despite the Kolmogorov-Smirnov test showing significant differences between the model output data and the observed (validation) data, we deemed the model with the utilized input parameters to be appropriate as the baseline model that could be used for

Table 4. Summary of what-if scenario evaluation

Scenarios and sub-scenarios	Implementation	Mean log ₁₀ cfu/mL		% of containers >20,000 cfu/mL at		The day when >34.32% of containers have >20,000 cfu/mL (actual % of containers with >20,000 cfu/mL) ¹
		d 7	d 10	d 7	d 10	
Baseline						
(1) Reduce frequency of post-pasteurization contamination PPC		3.59	4.68	34.32	56.18	7 (34.32%)
Increase periodic equipment cleaning (PEC; taking apart, cleaning, reassembling) of filler nozzles from once a week to twice a week.	Frequency of PPC reduced from 100% to 50%.	1.80	2.34	17.20	28.14	13 (35.59%)
Increase PEC of filler nozzles from once a week to twice a week and replace old filler nozzles.	Frequency of PPC reduced from 100% to 10%.	0.36	0.47	3.49	5.64	>14
(2) Reduce initial contamination concentration						
Institute improved preventative maintenance procedures; for example, standard operating procedures (SOP) and checklists to ensure appropriate frequency of replacement of O-rings, gaskets, mesh screens, rubber fittings, etc., assumed to yield 1-log reduced levels of PPC organisms in different possible niches.	Starting concentration is reduced by 1 log.	2.61	3.78	21.90	42.03	9 (35.35%)
Institute improved preventative maintenance procedures; for example, SOP and checklists to ensure appropriate frequency of replacement of O-rings, gaskets, mesh screens, rubber fittings, etc., assumed to yield 3-log reduced levels of PPC organisms in different possible niches. (Although 3-log reduction may be unrealistic under most circumstances, this provides insights into a theoretical best-case scenario.)	Starting concentration is reduced by 3 log.	-1.38	-0.48	7.67	17.71	>14
Use of antimicrobial coating on drains; coating is assumed to yield 2-log reduction of adherent PPC organisms.						
(3) Eliminate specific 16S rDNA sequence types of concern						
Perform "Seek and Destroy" targeting the subtype with the highest maximum growth rate [identify target 16S rDNA sequence type (ST), and use environmental sampling to eliminate source].						
<i>Outcome 1:</i> Seek and Destroy will eliminate any PPC occurring from this source (e.g., by equipment redesign).	Set frequency of contamination with ST 100 to 0. ²	3.22	4.28	32.47	53.83	8 (40.23%)
<i>Outcome 2:</i> Seek and Destroy will eliminate ST but will not change overall PPC frequency (simulating a situation where frequent PPC occurs and other sources and ST will continue to cause PPC).	Remove ST 100 from frequency table. ³	3.55	4.65	33.49	55.41	8 (41.47%)
Perform Seek and Destroy targeting the 2 subtypes that occur most frequently (identify these 2 ST and use environmental sampling to identify and eliminate source).						
<i>Outcome 1:</i> Seek and Destroy will eliminate any PPC occurring from the sources that harbored these 2 ST.	Set frequency of contamination with ST 13 and 9 to 0. ⁴	1.48	2.37	27.63	45.37	9 (40.00%)
<i>Outcome 2:</i> Seek and Destroy will eliminate ST but will not change overall PPC frequency (simulating a situation where frequent PPC occurs and other sources and ST will continue to cause PPC).	Remove ST 13 and 9 from frequency table. ⁵	3.56	4.64	33.74	55.56	8 (41.64%)

Continued

Table 4 (Continued). Summary of what-if scenario evaluation

Scenarios and sub-scenarios	Implementation	Mean log ₁₀ cfu/mL		% of containers >20,000 cfu/mL at		The day when >34.32% of containers have >20,000 cfu/mL (actual % of containers with >20,000 cfu/mL) ¹
		d 7	d 10	d 7	d 10	
(4) Improved temperature control Use sensors to indicate when a product goes above 6°C, such that the maximum temperature a product can experience is 6°C.	Temperature is truncated such that all containers cannot exceed a storage temperature of 6°C.	2.88	3.95	19.08	44.36	9 (36.09%)

¹34.32% represents the percentage of containers >20,000 cfu/mL at d 7 for the baseline model. This is the benchmark to determine how many additional days of shelf life each intervention would provide.

²ST 100 has the highest adjusted maximum growth rate. Setting the frequency of contamination of ST 100 to 0% means elimination of ST 100 would render any container that would have been contaminated with ST 100 as “uncontaminated.” This maintains the overall frequency of the remaining ST.

³Removing ST 100 from frequency table means containers that would have been contaminated with ST 100 are now contaminated with other, less frequent ST.

⁴ST 13 and 9 are the most frequent ST. Setting the frequency of contamination of ST 13 and 9 to zero means any container that would have been contaminated with ST 13 and 9 are rendered “uncontaminated.” This maintains the overall frequency of the remaining ST.

⁵Contamination with ST 13 and 9 are eliminated, which reduces the overall frequency of contamination. Containers that would have been contaminated with ST 13 and 9 are now contaminated with other, less frequent ST.

the sensitivity analyses and what-if scenario evaluation subsequently detailed. This approach is justified by the observation that differences between model predictions and observed data are minimal; for example, the mean model predicted and validation data count for d 7 were 4.8 log₁₀ cfu/mL and 4.3 log₁₀ cfu/mL, respectively, whereas the percentage of containers >20,000 cfu/mL SPC at d 7 were 66.5% and 59.1%, respectively. For d 10, the mean model predicted and validation data count were 6.3 and 6.4 log₁₀ cfu/mL, respectively, whereas the percentage of containers >20,000 cfu/mL SPC were 95.1% and 86.9%, respectively. The validated model predicted the mean counts for d 7, 10, and 14 as 3.6 log₁₀ cfu/mL, 4.7 log₁₀ cfu/mL, and 5.8 log₁₀ cfu/mL, respectively (Figure 3). The truncation on the right side of the d 10 and d 14 distributions can be explained by the ST reaching its N_{max} concentration.

Adjusted Maximum Growth Rate and Storage Temperature Have the Largest Effects on Improving the Overall Average Shelf Life of Pasteurized Fluid Milk

One-at-a-time sensitivity analyses of the 5 model input parameters (T , N_0 , F_{ST} , μ_{max_adj} , and t_{lag}) indicated that (1) adjusted maximum growth rate (μ_{max_adj}) and (2) storage temperature (T) had the largest effects on model outcomes, with the relative importance of these 2 parameters differing depending on whether changes were positive (i.e., an increase in temperature or an increase in adjusted maximum growth rate, both leading to increased spoilage due to PPC) or negative (i.e., a decrease in temperature or adjusted maximum growth rate). Specifically, storage temperature (T) appeared to affect outcomes more when values were increased, whereas adjusted maximum growth rate (μ_{max_adj}) appeared to be more influential when values were decreased (Figure 4). The parameters initial microbial concentration (N_0) and frequency (F_{ST}) showed an approximately equal influence on outcomes but were less important than adjusted maximum growth rate (μ_{max_adj}) and storage temperature (T ; Figure 4). Finally, lag phase (t_{lag}) appeared to have the least effect on model outcomes (Figure 4). For example, a 60% increase of lag time (t_{lag}), which will lead to reduced spoilage due to PPC, resulted in a minimal decrease in percentage of containers with >20,000 cfu/mL at d 7 from 34.32% to 31.25% (Figure 4A; Supplemental Table S1, <https://doi.org/10.7298/yth9-zq08>; Lau et al., 2021), whereas a reduction in adjusted maximum growth rate (μ_{max_adj}) by 60% (which also reduces spoilage due to PPC), resulted in a substantial decrease in percentage of containers with >20,000 cfu/mL at d 7 from 34.32% obtained with the baseline model to 4.97% (Figure 4A; see Supplemental

Table S1 for detailed data, Lau et al., 2021). Overall, sensitivity analysis findings were consistent regardless of whether analyses were performed using the percentage of containers with $>20,000$ cfu/mL on d 7 or d 10 as an outcome (Figure 4A and B).

Conducting What-If Scenarios Can Be Used to Evaluate the Effects of Applying Interventions

The model was used to evaluate what effects hypothetical what-if scenarios would have on predicted pasteurized fluid milk spoilage due to PPC. What-if scenarios were defined to capture possible interventions that would (1) reduce the frequency of PPC, (2) reduce the initial contamination concentration, (3) eliminate specific ST of concern, and (4) improve temperature control (Table 4). Metrics for effectiveness of interventions were the percentage of half-gallon containers with $>20,000$ cfu/mL at d 7 and 10, and the number of days of shelf life extension. These what-if analyses identified that scenario (1), reducing the frequency of PPC, had the greatest effect on increasing the shelf life of pasteurized milk, compared with the other 3 scenarios. Reducing the frequency of contamination from 100% to 50% predicted that 17.20% and 28.14% of half-gallon containers reached $>20,000$ cfu/mL on d 7 and 10, respectively (compared with 34.32% and 56.18% under the baseline model); reducing frequency of contamination to 10% predicted that 3.49% and 5.64% of half-gallon containers reached $>20,000$ cfu/mL on d 7 and 10, respectively. Using another sub-scenario, reducing the frequency of contaminated containers from 100% to 50% extended shelf life by 6 d, whereas reducing the frequency to 10% increased shelf life by >7 d (Table 4). By comparison, reducing the initial contamination level by 1 and 3 $\log_{10}$ [scenario (2); Table 4] reduced the percentage of containers with $>20,000$ cfu/mL to (1) 21.90% and 42.03% on d 7 and 10 (for 1 $\log_{10}$ reduction) and (2) 7.67% and 17.71% on d 7 and 10 (for 3 $\log_{10}$ reduction). Importantly, reducing initial contamination levels by 1 and 3 $\log_{10}$ yielded 0.82% and 28.80% of containers with starting inoculums of <1 cfu/container; our model was set to not allow bacterial growth in these containers, which is different from growth models that, by default, will allow for growth from levels below a single cell per container. Reducing initial contamination levels by 1 and 3 $\log_{10}$ extended shelf life by 2 and >7 d, respectively. The effect of reducing the contamination level by 2 $\log_{10}$ for 10% of containers was estimated to reduce spoilage due to PPC to 32.22% and 53.41% on d 7 and 10, respectively; this scenario simulates a “drain intervention” that reduces adhered bacteria in drains by 2 $\log_{10}$ (this includes the assumption that 10% of PPC can be traced back to drains as a source).

Figure 2. Density plot of the simulated concentration of post-pasteurization contamination (PPC) organisms ($\log_{10}$ cfu/mL) in fluid milk per half-gallon at (A) 7 d and (B) 10 d of storage at 6°C (solid line) and actual concentrations of PPC organisms in fluid milk per half-gallon at 7 d and 14 d of storage at 6°C, based on commercial fluid milk samples tested as part of the Cornell University Milk Quality Improvement Program Voluntary Shelf Life Program (VSL; dashed line). In (B) a peak occurs around 8 $\log_{10}$ cfu/mL for the simulated concentration of PPC organisms, which can be explained by the sequence type reaching its maximum microbial population (N_{max}) concentration.

Another set of interventions that were evaluated with our model were hypothetical Seek and Destroy interventions, which were designed either to target the fastest growing ST or to target the 2 most frequently isolates ST. For each of these 2 sub-scenarios, the effects of 2 possible outcomes were modeled, including (1) complete elimination of the targeted ST or ST where Seek and Destroy will completely eliminate contamination originating from these sources (therefore effectively reducing the percentage of containers with PPC), and (2) elimination of the targeted ST or ST with no change

Figure 3. Histograms of the simulated concentration of post-pasteurization contamination organisms in fluid milk per half-gallon over shelf life when stored at mean temperature of 4.06°C at (A) 7 d, (B) 10 d, and (C) 14 d. Monte Carlo simulations comprised 10,000 iterations and were based on 6 model parameters: (1) storage temperature (2) initial concentration of gram-negative bacteria in pasteurized milk (N_0), (3) plant pasteurized milk gram-negative bacteria sequence type frequency (F_{ST}), (4) maximum growth rate by sequence type (μ_{max_adj}), (5) lag phase by sequence type (t_{lag}), and (6) maximum microbial population by sequence type (N_{max}).

Figure 4. Sensitivity analyses assessing the effects of varying the parameters by intervals of 20% from -60 to 60 on the percent of half-gallons of fluid milk spoiled (>20,000 cfu/mL) on (A) 7 d and (B) 10 d of refrigerated storage for different aspects of post-pasteurization contamination (PPC) organism growth. Percent change of 0 represents the baseline model with which the other parameters were compared. Positive percent change represents a change to the parameter that will decrease shelf life or increase spoilage due to PPC. Negative percent change represents a change to the parameter that will increase shelf life or decrease spoilage due to PPC. (The results from the lag parameter were inverted to go in the same direction as the other parameters; e.g., for Figure 4A, the -60% change should not be interpreted as a 60% decrease in lag. The negative percent change represents a change in the parameter that will increase shelf life and thus should be interpreted as a 60% increase in shelf life; i.e., 60% increase in lag.)

in overall PPC frequency (simulating a situation where frequent PPC occurs, and other sources and ST will continue to cause PPC). Any of these sub-scenarios, regardless of which outcome was modeled, yielded increases in shelf life by only 1 to 2 d (Table 4). Similarly, modeling the influence of improving temperature control (in a way that would ensure that all containers would always be kept at $<6^{\circ}\text{C}$) extended shelf life only by an estimated 2 d (Table 4).

DISCUSSION

Although fluid milk spoilage due to PPC is well documented as a major challenge, not many data-driven tools are available to the dairy industry to help assess and identify appropriate and effective PPC interventions. To address this gap, we collected data on the growth characteristics of gram-negative bacteria associated with PPC, which were subsequently leveraged to develop a model that simulates pasteurized fluid milk spoilage due to PPC by predicting the concentration of PPC organisms in fluid milk over shelf life. Sensitivity analysis showed that storage temperature (T) and adjusted maximum growth rate ($\mu_{\text{max_adj}}$) had the greatest influence on the percentage of milk containers that showed bacterial counts above the threshold of 20,000 cfu/mL. Importantly, the developed model allowed for rapid simulation of what-if scenarios that can be used to evaluate the effects of different interventions on fluid milk shelf life; among the interventions modeled here, those that reduced the frequency of PPC contamination showed the greatest influence on shelf life.

A Wide Range of Gram-Negative Bacteria Can Grow in Skim Milk Broth at 6°C

The 17 isolates of gram-negative bacteria chosen to represent bacteria associated with PPC showed a wide range of lag phase duration. The selected isolates had estimated lag phase durations that ranged between less than 4 h and 34.9 h at 6°C . Among the 7 *Pseudomonas* spp. isolates characterized, estimated lag phase duration ranged from <4 to 25.5 h. This is consistent with reports from previous studies; for example, Chen et al. (2011) reported a lag phase of 9.4 h for *P. fluorescens* in whole UHT milk at 7°C , whereas Lin et al. (2016) reported a lag phase of 29.5 h for *P. fluorescens* in low-fat (1%) UHT milk at 4°C . Similarly, Stevenson et al. (2003) found that *P. fragi* in UHT skim milk at 4°C had an estimated lag phase duration of less than 24 h, whereas the lag phase duration for 4 different *P. fluorescens* ranged from 9.36 to 48.96 h. To allow us to compare our findings to those from these similar previous studies, we adjusted the previously reported lag

phases for *Pseudomonas* spp. (Stevenson et al., 2003; Chen et al., 2011; Lin et al., 2016) from the original study temperature (e.g., 4°C for Stevenson et al., 2003), to 6°C (the temperature we used in our growth experiments), using Ratkowsky's square root model (Ratkowsky et al., 1982); we found that the adjusted lag phase durations ranged between 0 and 31.6 h, which is comparable to results from our study. The remaining 10 non-*Pseudomonas* isolates that were tested in our study showed lag phase durations between <4 and 34.9 h. Unlike the growth data for *Pseudomonas* spp., growth data in milk for dairy-related non-*Pseudomonas* species is lacking in the literature. Although we observed differences in the duration of lag phases for different isolates, all of these recorded lag phases are considered short in relation to the duration of a typical milk shelf life; for example, shelf life of HTST-pasteurized milk with PPC ranges from 10 to 14 d at 6°C (Reichler et al., 2018).

Overall growth rates for the 17 gram-negative bacteria most commonly associated with PPC were between 0.058 to 0.141 ln cfu/h at 6°C , which represents doubling times ranging from 4.93 to 11.8 h. Of all isolates tested, *P. grimontii* ST 100 had the highest μ_{max} , at 0.141 ln cfu/h. The 2 most frequent ST in the data set, *Pseudomonas poae* and *P. fragi*, ST 9 and 13, had μ_{max} of 0.107 and 0.100 ln cfu/h, respectively. These growth rates are lower compared with the growth rates reported by Stevenson et al. (2003), who found that in UHT skim milk at 4°C , *P. fragi* had a growth rate of approximately 0.136 ln cfu/mL, and 4 *P. fluorescens* isolates had growth rates in the range of 0.120 to 0.142 ln cfu/mL. Similarly lower growth rates were determined for all 7 *Pseudomonas* spp. (mean of 0.107 ln cfu/h, range of 0.086–0.141 ln cfu/h) and 10 non-*Pseudomonas* spp. isolates (mean of 0.092 ln cfu/h, range of 0.058–0.133 ln cfu/h) characterized during this study. More rapid growth rates of *Pseudomonas* spp. compared with non-*Pseudomonas* spp. are consistent with prior studies that found that *Pseudomonas* spp. are more frequently responsible for milk spoilage compared with other gram-negative organisms (i.e., *Enterobacteriaceae*; Ternström et al., 1993; Ranieri and Boor, 2009).

PPC Models Using Growth Parameters from Pure Culture in Skim Milk Broth Require an Adjustment Factor to Better Predict PPC

Initial validation of the PPC model developed here showed that the simulation model consistently overestimated the percentage of spoiled half-gallons on d 7 and 10 of shelf life. We reasoned that this was most likely due to the fact that growth data were collected in SMB, which may allow for more rapid growth than HTST milk. For example, presence of residual lacto-

peroxidase in HTST milk (which would be absent from SMB, which has undergone substantially harsher heat treatment than HTST milk) could partially inhibit growth of bacteria introduced by PPC (Reiter and Härnqvist, 1984; Munsch-Alatossava et al., 2018). Lactoperoxidase plays a bactericidal role due to its ability to form oxidized compounds with antimicrobial properties (Munsch-Alatossava et al., 2018). Alternatively, or additionally, bacterial growth in actual commercial milk with PPC may also be slower compared with the growth determined in this study, since growth data in SMB were collected in pure culture, whereas commercial milk samples are likely contaminated with multiple bacterial subtypes. This is supported by Reichler et al. (2018), who found that, among 280 HTST fluid milk samples from 10 different processing plants, samples that showed SPC >20,000 cfu/mL in at least one day of shelf life had a mean of 8.5 different bacterial isolates (based on 16S sequencing). Another study that collected pasteurized milk samples from 4 processing plants also reported considerable genetic diversity of *Pseudomonas* spp. within fluid milk products, which further supports that PPC may often represent contamination with multiple strains (Dogan and Boor, 2003). Competition for limited nutrients or production of inhibitory substances by some strains (Gram et al., 2002) can explain why growth in pure cultures is faster than bacterial growth in real-life milk samples. Similarly, Xu (2021) emphasized the importance of interactions in mixed cultures, including possible coexisting interactions, where species may slow one another's growth. To address the fact that the initial model overestimated bacterial growth, we calculated an adjustment factor that adjusted the growth rates determined in SMB (0.098 ln cfu/h, which corresponds to a doubling time of 7.1 h) to the growth rates that are based on available growth data expected in pasteurized milk (0.067 ln cfu/h, which represents a doubling time of 10.3 h). Applying the adjustment factor results in a change in the test statistic of the Kolmogorov-Smirnov test from 0.65 and 0.40 for the initial model without the adjustment factor, to 0.18 and 0.24 for the model with the adjustment factor, for d 7 and 10, respectively. Once the adjustment factor was implemented in the PPC model, the model was able to more accurately predict the percentage of spoiled half-gallons for commercial milk containers.

Importantly, adjustment factors have been used in previous studies to account for certain limitations in predictive models and to tune models to predict observed real-world outcomes. For example, in a risk assessment of *Listeria monocytogenes* in ready-to-eat foods, researchers used an adjustment factor to account for the variability of the parameters that influence the relationship between the dose and illness severity (FDA,

2003); this adjustment factor was necessary because the lethal dose was determined using mortality data from mice studies, and adjustment was made based on Centers for Disease Control and Prevention estimates of annual death rates (FDA, 2003). Another adjustment factor was used in the risk assessment to account for overestimation of *L. monocytogenes* prevalence in ready-to-eat foods from older studies compared with recent studies that could be a result of higher actual contamination levels or nonrepresentative sampling (FDA, 2003). These adjustment factors are used to account for studies conducted with experimental animals and the limited amount of data available, respectively. Although these previous applications of adjustment factors validate our use of an adjustment factor, future experiments to identify the reason for why SMB-based growth parameters apparently overestimate fluid milk spoilage will be important, particularly as they may help identify improvements that may be needed for future models (e.g., inclusion of multiple strain interactions instead of the simplifying assumption that a given container will be contaminated with only 1 ST).

Adjusted Maximum Growth Rate and Storage Temperature Have the Greatest Influence on Predicted Concentrations of Bacteria Associated with PPC in Fluid Milk Over Shelf Life

Sensitivity analysis indicated that adjusted maximum growth rate ($\mu_{\max_adj}$) and storage temperature (T) have the strongest effects on predicted percentage of containers spoiled due to PPC on d 7 and 10 of shelf life, suggesting that accurate and sufficient data for these 2 parameters are important for the type of spoilage models developed here. The importance of maximum growth rate for estimating microbial spoilage and bacterial growth was also demonstrated by Buehler et al. (2018) using the Monte Carlo simulation model they developed to predict pasteurized fluid milk spoilage due to psychrotolerant spore-forming bacteria. Sensitivity analysis of a model that was developed to predict growth of *L. monocytogenes* on cheese curds also found that maximum growth rate was among the most influential parameters (Couvert et al., 2010). Similarly, in a publication that reported a general framework for quantitative microbial food safety risk assessment (McNab, 1998), maximum growth rate (designated as "growth rate after treatment") was consistently found to be one of the top 3 ranked parameters across sensitivity analyses conducted for different specific scenarios. Likewise, previous studies have also found storage temperature to have a considerable effect on the output of different microbial risk assessment models. For example, a Monte Carlo simulation model for

bacterial spoilage of fluid milk developed by Schaffner et al. (2003) predicted that decreasing storage temperature from 6.5°C to 4.4°C resulted in a reduction of spoilage (defined as samples exceeding 10^7 cfu/mL) due to growth of psychrotolerant organisms from 67% to 28% by d 14. Similarly, the Monte Carlo simulation model for fluid milk spoilage due to psychrotolerant spore-formers, developed by Buehler et al. (2018), predicted that reducing refrigeration temperature from 6°C to 4°C would result in a reduction of spoilage at d 21 (defined as samples exceeding 20,000 cfu/mL) from 66% to 9%. The sensitivity analysis and previous studies suggest a need to place more emphasis on gathering more accurate data on maximum growth rate and storage temperature when using models to predict microbial growth.

What-If Scenarios Can Be Used to Assess the Effects of Different Interventions on Shelf Life

The model developed here provided an opportunity to run different “what-if” (or scenario) analyses; these analyses provide an opportunity to assess the influence different intervention strategies may have on product shelf life, potentially helping industry with decision-making. The what-if analyses we conducted indicated that reducing the frequency of PPC contamination could substantially extend the shelf life of pasteurized fluid milk (e.g., increase in shelf life by 6 d if PPC frequency is reduced from 100% to 50%). Possible interventions that would be expected to reduce the frequency of contamination would be implementation of enhanced periodic equipment cleaning procedures on key equipment responsible for PPC, such as filler nozzles, or replacement of equipment or equipment components with more hygienic and easier-to-clean counterparts (e.g., replacement of poorly designed fillers, replacement of difficult-to-clean filler nozzles). The potential positive effects of these types of interventions are supported by Reichler et al. (2020) and Ralyea et al. (1998), who showed that an improved preventative maintenance program targeting rubber parts of a specific filler decreased the frequency of PPC in fluid milk containers. Additionally, Murphy et al. (2021) found that having well-designed cleaning and sanitation programs, along with appropriate training, are among the top factors necessary to reduce PPC.

Reducing the initial concentration of PPC organisms was also predicted to substantially extend the shelf life of fluid milk if reductions in initial PPC contamination levels were large (e.g., a 3 $\log_{10}$ reduction increased shelf life by >7 d, whereas a 1 $\log_{10}$ reduction increased shelf life by only 2 d). Although a 3 $\log_{10}$ reduction in initial concentration may be unrealistic under most

circumstances, this analysis provides a best-case scenario for the effects of interventions that reduce initial PPC contamination levels. Interventions that decrease initial contamination levels may include instituting improved preventative maintenance procedures such as using standard operating procedures and checklists to ensure appropriate frequency of replacement of equipment parts such as O-rings, gaskets, mesh screens, and rubber fittings. Although very limited data on actual effects of specific interventions on initial PPC levels are available, one previous study did indicate that improved inspection and cleaning, as well as preventative maintenance programs, can increase the reliability of a pizza processing line and thus reduce the frequency of failures due to equipment and lead to improvement in product quality (Tsarouhas, 2007). Importantly, a quality cost model for processing plants developed by Zugarramurdi et al. (2007) also indicated that implementation of a quality assurance plan and preventative maintenance, including replacement and maintenance of equipment parts, are appropriate and economically viable strategies to improve product quality.

Although interventions that reduce frequency and levels of PPC contamination were shown to have substantial effects on enhancing average fluid milk shelf life, other strategies tested in what-if scenarios were less effective at extending shelf life. For example, eliminating specific spoilage organisms or organism sources was predicted to extend shelf life by only 1 or 2 d (assuming that these interventions would not decrease PPC levels or frequency). Even with the limited effects identified by our model, Seek and Destroy-type strategies to eliminate sources of PPC could still be valuable, if these efforts are able to decrease PPC levels or frequency. For example, previous studies have shown that Seek and Destroy interventions can successfully reduce *L. monocytogenes* contamination of food products from environmental sources (Malley et al., 2015). A what-if scenario also suggested that ensuring improved temperature controls (defined here as ensuring that no fluid milk containers would be exposed to >6°C) extended shelf life by only 2 d. This represents a potentially important finding, in part because temperature is one of the many food quality indicators (e.g., gas production, pH change, humidity) that can be targeted using intelligent packaging (Fuentes et al., 2016; Yousefi et al., 2019). For example, Ellouze and Augustin (2010) evaluated a biological time-temperature indicator that could successfully be used as a quality and safety indicator for ground beef and spiced cooked chicken slices under modified atmosphere across shelf life. Importantly, although our data suggested that limited average shelf life enhancement could be achieved by assuring product distribution at <6°C, future work could use our model

to evaluate different temperature control strategies and their effects on shelf life. Influence of time-temperature indicators could also be more pronounced if the baseline temperature distribution included more abusive temperatures than the baseline used in our model. Importantly, our analysis only assessed spoilage due to PPC and did not assess other potential benefits of this type of indicators, such as reduced growth of *L. monocytogenes*, which represents a food safety hazard for which improved temperature control has been well established to reduce food safety risks (Walker et al., 1990; Chan and Wiedmann, 2008).

Although the what-if analyses conducted here provide initial insights on the effects of various intervention strategies on fluid milk shelf life, the model developed here has some limitations that will need to be addressed to enhance its value, particularly as a decision-support tool for industry. Namely, although the model developed here provides the ability to assess the potential effects of different interventions on fluid milk shelf life, additional features to facilitate decision-making should be incorporated; for example, incorporation of a cost-benefit analysis module would help industry assess the economic feasibility and return on investment for different interventions. Additionally, an alternative approach can be used for the interpretation of containers assigned an initial concentration less than 1 cfu per half-gallon. Concentrations below 1 cfu per half-gallon can be interpreted as probabilities; for example, if the assigned concentration was 0.1 cfu per half-gallon, that container would have a 10% probability of containing 1 cfu. However, this interpretation would require a change to the model structure, a process of reassigning the initial concentration with at least 1 cfu per half-gallon, and would increase the complexity of the model. Importantly, the model developed here only considers fluid milk spoilage due to PPC, yet contamination with gram-positive spore-forming bacteria can also lead to spoilage later in shelf life, which should be considered when assessing potential intervention strategies for potential influence on shelf life. For example, 2 intervention strategies evaluated here, reducing frequency of PPC from 100% to 10% and reducing the initial contamination by 3 log₁₀, predicted that shelf life would be extended by greater than 7 d. When evaluating the what-if scenarios that extend shelf life by greater than 7 d, it would be advisable to evaluate the effects using both the current model that predicts spoilage by PPC and the previously developed spoilage model (Buehler et al., 2018) that predicts spoilage by aerobic psychrotolerant spore-formers. A future combined model would be valuable to evaluate the effects of both gram-positive spore-forming bacteria and PPC organisms.

CONCLUSIONS

Overall, the model developed here, along with other models discussed, not only represents a starting point for digital tools to help industry identify appropriate approaches for enhancing fluid milk quality, but also represents a blueprint for the type of decision-support tools that can be developed and applied to other dairy products. Importantly, these types of tools will allow for more cost-effective approaches to identify appropriate shelf life extension strategies and will reduce the need for expensive field trials. Finally, the tools developed here can also be used to estimate shelf life of fluid milk and ultimately other dairy products, which provide opportunities for improved shelf life labeling, including dynamic shelf life labeling.

ACKNOWLEDGMENTS

Funding for this project was provided by the Foundation for Food and Agriculture Research (FFAR, Washington, DC; award no. CA18-SS-0000000206) and by the New York State Milk Promotion Advisory Board (Albany, NY), which funds the VSL program. The authors thank the students, faculty, and staff of the Cornell University Milk Quality Improvement Program (Ithaca, NY), with special thanks to Samuel Reichler. The authors have not stated any conflicts of interest.

REFERENCES

- Alles, A. A., M. Wiedmann, and N. H. Martin. 2018. Rapid detection and characterization of postpasteurization contaminants in pasteurized fluid milk. *J. Dairy Sci.* 101:7746–7756. <https://doi.org/10.3168/jds.2017-14216>.
- Arnold, T., and J. W. Emerson. 2013. dgof: Discrete Goodness-of-Fit Tests. Accessed Nov. 23, 2020. <https://cran.r-project.org/web/packages/dgof/dgof.pdf>.
- Baranyi, J., and T. A. Roberts. 1994. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.* 23:277–294. [https://doi.org/10.1016/0168-1605\(94\)90157-0](https://doi.org/10.1016/0168-1605(94)90157-0).
- Baty, F., and M. Delignette-Muller. 2017. nlsMicrobio: Data sets and nonlinear regression models dedicated to predictive microbiology. Accessed Nov. 23, 2021. <https://cran.r-project.org/web/packages/nlsMicrobio/nlsMicrobio.pdf>.
- Baty, F., C. Ritz, S. Charles, M. Brutsche, J.-P. Flandrois, and M.-L. Delignette-Muller. 2015. A toolbox for nonlinear regression in R: The package nlstools. *J. Stat. Softw.* 66:1–21. <https://doi.org/10.18637/jss.v066.i05>.
- Buchanan, R. L., R. C. Whiting, and W. C. Damert. 1997. When is simple good enough: A comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. *Food Microbiol.* 14:313–326. <https://doi.org/10.1006/fmic.1997.0125>.
- Buehler, A. J., N. H. Martin, K. J. Boor, and M. Wiedmann. 2018. Psychrotolerant spore-former growth characterization for the development of a dairy spoilage predictive model. *J. Dairy Sci.* 101:6964–6981. <https://doi.org/10.3168/jds.2018-14501>.
- Butts, J. 2003. Seek & destroy: Identifying and controlling *Listeria monocytogenes* growth niches. Accessed May 15, 2021. <https://www.food-safety.com/articles/4802-seek-destroy-identifying-and-controlling-listeria-monocytogenes-growth-niches>.

- Buzby, J. C., H. Farah-Wells, and J. Hyman. 2014. The estimated amount, value, and calories of postharvest food losses at the retail and consumer levels in the United States. USDA-ERS Economic Information Bulletin Number 121.
- Chan, Y. C., and M. Wiedmann. 2008. Physiology and genetics of *Listeria monocytogenes* survival and growth at cold temperatures. *Crit. Rev. Food Sci. Nutr.* 49:237–253. <https://doi.org/10.1080/10408390701856272>.
- Chen, T. R., Q. K. Wei, and Y. J. Chen. 2011. *Pseudomonas* spp. and *Hafnia alvei* growth in UHT milk at cold storage. *Food Control* 22:697–701. <https://doi.org/10.1016/j.foodcont.2010.10.004>.
- Couvert, O., A. Pinon, H. Bergis, F. Bourdichon, F. Carlin, M. Cornu, C. Denis, N. Gnanou Besse, L. Guillier, E. Jamet, E. Mettler, V. Stahl, D. Thuault, V. Zuliani, and J.-C. Augustin. 2010. Validation of a stochastic modelling approach for *Listeria monocytogenes* growth in refrigerated foods. *Int. J. Food Microbiol.* 144:236–242. <https://doi.org/10.1016/j.ijfoodmicro.2010.09.024>.
- 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. <https://doi.org/10.1128/AEM.69.1.130-138.2003>.
- Ellouze, M., and J. C. Augustin. 2010. Applicability of biological time temperature integrators as quality and safety indicators for meat products. *Int. J. Food Microbiol.* 138:119–129. <https://doi.org/10.1016/j.ijfoodmicro.2009.12.012>.
- FAO. 2010. Greenhouse gas emissions from the dairy sector: A life cycle assessment. UN FAO.
- FAO. 2019. The State of Food and Agriculture 2019. Moving forward on food loss and waste reduction. UN FAO.
- FDA. 2003. Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Accessed Nov. 23, 2021. <https://www.fda.gov/media/124721/download>.
- FDA. 2019. Standards for grade “A” milk and/or milk products. Pages 32–35 in Grade “A” Pasteurized Milk Ordinance. US Department of Health and Human Services, Public Health Service.
- Fuertes, G., I. Soto, R. Carrasco, M. Vargas, J. Sabattin, and C. Lagos. 2016. Intelligent packaging systems: Sensors and nanosensors to monitor food quality and safety. *J. Sens.* 2016:4046061. <https://doi.org/10.1155/2016/4046061>.
- Garrido-Sanz, D., J. P. Meier-Kolthoff, M. Goker, M. Martin, R. Rivilla, and M. Redondo-Nieto. 2016. Genomic and genetic diversity within the *Pseudomonas fluorescens* complex. *PLoS One* 11:e0150183. <https://doi.org/10.1371/journal.pone.0150183>.
- Gram, L., L. Ravn, R. Rasch, J. B. Bruhn, A. B. Christensen, and M. Givskov. 2002. Food spoilage—Interactions between food spoilage bacteria. *Int. J. Food Microbiol.* 78:79–97. [https://doi.org/10.1016/S0168-1605\(02\)00233-7](https://doi.org/10.1016/S0168-1605(02)00233-7).
- Gruetzmacher, T. J., and R. L. Bradley Jr.. 1999. Identification and control of processing variables that affect the quality and safety of fluid milk. *J. Food Prot.* 62:625–631. <https://doi.org/10.4315/0362-028X-62.6.625>.
- Hoover, D. 2017. Estimating quantities and types of food waste at the city level. National Resources Defense Council.
- Kang, Y.-J., and J. F. Frank. 1990. Characteristics of biological aerosols in dairy processing plants. *J. Dairy Sci.* 73:621–626. [https://doi.org/10.3168/jds.S0022-0302\(90\)78712-7](https://doi.org/10.3168/jds.S0022-0302(90)78712-7).
- Lau, S., A. Trmcic, N. H. Martin, M. Wiedmann, and S. I. Murphy. 2021. Development of a Monte Carlo simulation model to predict pasteurized fluid milk spoilage due to post-pasteurization contamination with gram-negative bacteria_Supplemental Material. <https://doi.org/10.7298/yth9-zq08>.
- Lin, H., M. Shavezipur, A. Yousef, and F. Maleky. 2016. Prediction of growth of *Pseudomonas fluorescens* in milk during storage under fluctuating temperature. *J. Dairy Sci.* 99:1822–1830. <https://doi.org/10.3168/jds.2015-10179>.
- Malley, T. J. V., J. Butts, and M. Wiedmann. 2015. Seek and Destroy Process: *Listeria monocytogenes* process controls in the ready-to-eat meat and poultry industry. *J. Food Prot.* 78:436–445. <https://doi.org/10.4315/0362-028X.JFP-13-507>.
- Martin, N. H., K. J. Boor, and M. Wiedmann. 2018. Symposium review: Effect of post-pasteurization contamination on fluid milk quality. *J. Dairy Sci.* 101:861–870. <https://doi.org/10.3168/jds.2017-13339>.
- Martin, N. H., N. Carey, S. Murphy, D. Kent, J. Bang, T. Stubbs, M. Wiedmann, and R. Dando. 2016. Exposure of fluid milk to LED light negatively affects consumer perception and alters underlying sensory properties. *J. Dairy Sci.* 99:4309–4324. <https://doi.org/10.3168/jds.2015-9603>.
- Martin, N. H., N. R. Carey, S. C. Murphy, M. Wiedmann, and K. J. Boor. 2012. A decade of improvement: New York State fluid milk quality. *J. Dairy Sci.* 95:7384–7390. <https://doi.org/10.3168/jds.2012-5767>.
- Mazerolle, M. 2020. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). Accessed Nov. 23, 2021. <https://cran.r-project.org/web/packages/AICcmodavg/AICcmodavg.pdf>.
- McNab, W. B. 1998. A general framework illustrating an approach to quantitative microbial food safety risk assessment. *J. Food Prot.* 61:1216–1228. <https://doi.org/10.4315/0362-028X-61.9.1216>.
- Munsch-Alatossava, P., O. Gursoy, P. M. Lorilla, J.-P. Gauchi, and T. Alatossava. 2018. Chapter 15: Antibacterial effects and modes of action of the activated lactoperoxidase system (LPS), of CO₂ and N₂ gas as food-grade approaches to control bovine raw milk-associated bacteria. Pages 519–541 in *Food Control and Biosecurity*. A. M. Holban and A. M. Grumezescu, ed. Academic Press.
- Murphy, S. I., S. J. Reichler, N. H. Martin, K. J. Boor, and M. Wiedmann. 2021. Machine learning and advanced statistical modeling can identify key quality management practices that affect post-pasteurization contamination of fluid milk. *J. Food Prot.* 84:1496–1511. <https://doi.org/10.4315/JFP-20-431>.
- Pouillot, R., M. B. Lubran, S. C. Cates, and S. Dennis. 2010. Estimating parametric distributions of storage time and temperature of ready-to-eat foods for US households. *J. Food Prot.* 73:312–321. <https://doi.org/10.4315/0362-028X-73.2.312>.
- R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing.
- Ralyea, R. D., M. Wiedmann, and K. J. Boor. 1998. Bacterial tracking in a dairy production system using phenotypic and ribotyping methods. *J. Food Prot.* 61:1336–1340. <https://doi.org/10.4315/0362-028X-61.10.1336>.
- Ranieri, M. L., and K. J. Boor. 2009. Short communication: Bacterial ecology of high-temperature, short-time pasteurized milk processed in the United States. *J. Dairy Sci.* 92:4833–4840. <https://doi.org/10.3168/jds.2009-2181>.
- Ratkowsky, D. A., J. Olley, T. A. McMeekin, and A. Ball. 1982. Relationship between temperature and growth rate of bacterial cultures. *J. Bacteriol.* 149:1–5. <https://doi.org/10.1128/jb.149.1.1-5.1982>.
- Reichler, S. J., S. I. Murphy, A. W. Erickson, N. H. Martin, A. B. Snyder, and M. Wiedmann. 2020. Interventions designed to control postpasteurization contamination in high-temperature, short-time-pasteurized fluid milk processing facilities: A case study on the effect of employee training, clean-in-place chemical modification, and preventive maintenance programs. *J. Dairy Sci.* 103:7569–7584. <https://doi.org/10.3168/jds.2020-18186>.
- Reichler, S. J., A. Trmcic, N. H. Martin, K. J. Boor, and M. Wiedmann. 2018. *Pseudomonas fluorescens* group bacterial strains are responsible for repeat and sporadic postpasteurization contamination and reduced fluid milk shelf life. *J. Dairy Sci.* 101:7780–7800. <https://doi.org/10.3168/jds.2018-14438>.
- Reiter, B., and G. Härmulv. 1984. Lactoperoxidase antibacterial system: Natural occurrence, biological functions and practical applications. *J. Food Prot.* 47:724–732. <https://doi.org/10.4315/0362-028X-47.9.724>.
- Schaffner, D., S. Duffy, R. Montville, and S. Smith. 2003. Monte Carlo simulation of the shelf life of pasteurized milk as affected by temperature and initial concentration of spoilage organisms. *Food Prot. Trends* 23:1014–1021.
- Schröder, M. J. A. 1984. Origins and levels of post pasteurization contamination of milk in the dairy and their effects on keep-

- ing quality. J. Dairy Res. 51:59–67. <https://doi.org/10.1017/S0022029900023323>.
- Stevenson, R. G., M. T. Rowe, G. B. Wisdom, and D. Kilpatrick. 2003. Growth kinetics and hydrolytic enzyme production of *Pseudomonas* spp. isolated from pasteurized milk. J. Dairy Res. 70:293–296. <https://doi.org/10.1017/S0022029903006204>.
- Sunga, F. C. A., D. R. Heldman, and T. I. Hedrick. 1970. Microorganisms from arms and hands of dairy plant workers. J. Milk Food Technol. 33:178–181. <https://doi.org/10.4315/0022-2747-33.5.178>.
- Ternström, A., A.-M. Lindberg, and G. Molin. 1993. Classification of the spoilage flora of raw and pasteurized bovine milk, with special reference to *Pseudomonas* and *Bacillus*. J. Appl. Bacteriol. 75:25–34. <https://doi.org/10.1111/j.1365-2672.1993.tb03403.x>.
- Tsarouhas, P. 2007. Implementation of total productive maintenance in food industry: A case study. J. Qual. Mainten. Eng. 13:5–18. <https://doi.org/10.1108/13552510710735087>.
- Vangay, P., E. B. Fugett, Q. Sun, and M. Wiedmann. 2013. Food microbe tracker: A web-based tool for storage and comparison of food-associated microbes. J. Food Prot. 76:283–294.
- Vinutha, H. P., B. Poornima, and B. M. Sagar. 2018. Detection of outliers using interquartile range technique from intrusion dataset. Pages 511–518 in Information and Decision Sciences. S. Satapathy, J. Tavares, V. Bhateja, and J. Mohanty, ed. Springer Singapore.
- Walker, S. J., P. Archer, and J. G. Banks. 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. J. Appl. Bacteriol. 68:157–162. <https://doi.org/10.1111/j.1365-2672.1990.tb02561.x>.
- Werner, B. G., J. Y. Wu, and J. M. Goddard. 2019. Antimicrobial and antifouling polymeric coating mitigates persistence of *Pseudomonas aeruginosa* biofilm. Biofouling 35:785–795. <https://doi.org/10.1080/08927014.2019.1660774>.
- Xu, P. 2021. Dynamics of microbial competition, commensalism, and cooperation and its implications for coculture and microbiome engineering. Biotechnol. Bioeng. 118:199–209. <https://doi.org/10.1002/bit.27562>.
- Yousefi, H., H.-M. Su, S. M. Imani, K. Alkhalidi, C. D. M. Filipe, and T. F. Didar. 2019. Intelligent food packaging: A review of smart sensing technologies for monitoring food quality. ACS Sens. 4:808–821. <https://doi.org/10.1021/acssensors.9b00440>.
- Zugarramurdi, A., M. A. Parin, L. Gadaleta, and H. M. Lupin. 2007. A quality cost model for food processing plants. J. Food Eng. 83:414–421. <https://doi.org/10.1016/j.jfoodeng.2007.03.029>.
- Zwietering, M. H., J. T. de Koos, B. E. Hasenack, J. C. de Witt, and K. van't Riet. 1991. Modeling of bacterial growth as a function of temperature. Appl. Environ. Microbiol. 57:1094–1101. <https://doi.org/10.1128/aem.57.4.1094-1101.1991>.

ORCIDS

- S. Lau <https://orcid.org/0000-0001-9052-5094>
 A. Trmcic <https://orcid.org/0000-0002-2249-5839>
 N. H. Martin <https://orcid.org/0000-0003-1704-0634>
 M. Wiedmann <https://orcid.org/0000-0002-4168-5662>
 S. I. Murphy <https://orcid.org/0000-0001-9092-0625>