A standard bacterial isolate set for research on contemporary dairy spoilage

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ABSTRACT

Food spoilage is an ongoing issue that could be dealt with more efficiently if some standardization and unification was introduced in this field of research. For example, research and development efforts to understand and reduce food spoilage can greatly be enhanced through availability and use of standardized isolate sets. To address this critical issue, we have assembled a standard isolate set of dairy spoilers and other selected nonpathogenic organisms frequently associated with dairy products. This publicly available bacterial set consists of (1) 35 gram-positive isolates including 9 *Bacillus* and 15 *Paenibacillus* isolates and (2) 16 gram-negative isolates including 4 *Pseudomonas* and 8 coliform isolates. The set includes isolates obtained from samples of pasteurized milk ($n = 43$), pasteurized chocolate milk ($n = 1$), raw milk ($n = 1$), cheese ($n = 2$), as well as isolates obtained from samples obtained from dairy-powder production ($n = 4$). Analysis of growth characteristics in skim milk broth identified 16 gram-positive and 13 gram-negative isolates as psychrotolerant. Additional phenotypic characterization of isolates included testing for activity of β -galactosidase and lipolytic and proteolytic enzymes. All groups of isolates included in the isolate set exhibited diversity in growth and enzyme activity. Source data for all isolates in this isolate set are publicly available in the FoodMicrobeTracker database (<http://www.foodmicrobetracker.com>), which allows for continuous updating of information and advancement of knowledge on dairy-spoilage representatives included in this isolate set. This isolate set along with publicly available isolate data provide a unique resource that will help advance knowledge of dairy-spoilage organisms as well as aid industry in development and validation of new control strategies.

Key words: isolate set, dairy spoilage, fluid milk, dairy powder, cheese

INTRODUCTION

Dairy processors, together with the rest of the food industry, are striving toward extending the shelf-life

of their products while following the modern trends of minimal processing. Despite the advances in food handling and processing, a large amount of milk and dairy products are still being wasted every year. The USDA Economic Research Service (USDA, 2012) estimates loss to be more than 29% for fluid and fermented milk and more than 27% for cheese. Although a large proportion of these losses appears to occur at the consumer and retail level (Golan and Buzby, 2015), microbial spoilage appears to represent an important contributor to these losses (Remenant et al., 2015).

Spoilage microorganisms in fluid milk are usually responsible for 2 types of spoilage. One type of spoilage is typically associated with postpasteurization contamination (PPC) and leads to rapid spoilage of fluid milk within 7 to 14 d. Most postpasteurization contaminants that lead to fluid-milk spoilage represent psychrotolerant gram-negative bacteria, including *Pseudomonas* species, coliforms, and other members of the *Enterobacteriaceae* family (Boor, 2001; Martin et al., 2012a). With advances in process control and reduction in occurrence of PPC, spoilage of fluid milk due to outgrowth of aerobic, gram-positive, sporeforming bacteria has become more apparent. This type of spoilage is caused by different psychrotolerant bacilli, which mainly represent the genus *Paenibacillus* and some *Bacillus* species such as *B. weihenstephanensis* (Ranieri and Boor, 2009; Ivy et al., 2012; Ranieri et al., 2012). Spoilage due to these organisms typically occurs around 17 to 21 d of shelf-life if milk is stored around 6°C. Both sporeforming and nonsporeforming gram-positive bacteria are also occasionally involved in PPC. In this type of spoilage the onset can be more rapid, suggesting contamination with viable bacterial cells, which are not delayed by the germination process of spores.

Aerobic, gram-positive, sporeforming bacteria are also important contaminants of dairy powders. High temperatures involved in production of dairy powders select for thermophilic bacilli, which can influence the stability of dairy powders and reconstituted dairy powders. The majority of isolates obtained from different dairy powders belong to 1 of 3 members of family *Bacillaceae*, including *Anoxybacillus flavithermus*, *Geobacillus stearothermophilus*, and *Bacillus licheniformis*. Besides these 3 most prevalent species, dairy powders

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can also contain other species and genera from this family (Ronimus et al., 2003; Rückert et al., 2004; Watterson et al., 2014).

Bacterial contaminants also play an important role in cheese spoilage. Similarly to fluid milk, psychrotolerant, gram-negative bacteria, such as *Pseudomonas* and coliform bacteria, are known to cause spoilage of cottage cheese and other fresh dairy products (Lemieux and Simard, 1992; Martin et al., 2011a). Coliform bacteria are also known to cause several defects in hard-type cheese, including early blowing. Late blowing most often associated with Swiss-type cheese is caused by *Clostridium tyrobutyricum*, an anaerobic, gram-positive, sporeforming bacterium (Cogan and Beresford, 2002).

Different isolate sets and reference isolate sets are a well-established resource in research and development. An early example of such an isolate set is the *Escherichia coli* diversity collection developed by Ochman and Selander in 1984 (Ochman and Selander, 1984). Since then other isolate sets have been introduced, which are mostly capturing isolate diversity within a specific pathogen group, such as *Salmonella* (Boyd et al., 1993), *Listeria monocytogenes* (Fugett et al., 2006), *Cronobacter* (Ivy et al., 2013), and as the most recent example, *Clostridium difficile* (Janezic et al., 2014). Isolate sets like these are used in development of new detection methods and control measures as well as studies that characterize a given group of microorganisms. The success of different pathogen isolate sets and collections could be extended from pathogenic bacteria to also other microbial groups of interest, such as spoilage bacteria.

The objective of this study was to assemble and offer a publicly available isolate set of contemporary bacterial isolates that are known to cause or have the potential to cause spoilage of fluid milk and dairy products. We anticipate that this isolate set together with the availability of updated isolate data, through the FoodMicrobeTracker database (formerly known as Pathogen Tracker; Vangay et al., 2013), will provide a valuable tool for studying bacterial dairy spoilers as well as provide the means for unification and faster progress in dairy research and technology.

MATERIALS AND METHODS

Isolates

Isolates included in this isolate set of dairy spoilers are listed in the Table 1 and were obtained from a large collection of isolates that have been collected as part of several dairy-quality research and surveillance projects conducted by Cornell's Milk Quality Improvement Pro-

gram. The complete isolate set consists of 51 individual isolates, 35 isolates representing gram-positive and 16 isolates representing gram-negative bacteria. The isolates originated from different samples of pasteurized milk ($n = 43$), pasteurized chocolate milk ($n = 1$), raw milk ($n = 1$), cheese ($n = 2$), and also from samples obtained from dairy-powder production ($n = 4$). Although the majority of these isolates are described here for the first time, 20 isolates have been described in previously published studies (Huck et al., 2007b, 2008; Ranieri and Boor, 2009; Ranieri et al., 2009; Martin et al., 2011a; Ivy et al., 2012; Van Tassell et al., 2012; Moreno Switt et al., 2014; Watterson et al., 2014). Isolates are stored at -80°C in brain heart infusion (BHI) broth (Difco, BD, Sparks, MD) with 15% glycerol as part of culture collection maintained by the Food Safety Laboratory (FSL), Department of Food Science, Cornell University, Ithaca, New York.

Identification by DNA Sequencing–Based Methods

All isolates included in the isolate set were identified by sequencing part of the 16S rDNA or *rpoB* or both. Sequencing of *rpoB* was used to identify isolates from the family *Bacillaceae*, as previously described (Huck et al., 2007a; Ivy et al., 2012). Briefly, *rpoB* data were initially used to classify isolates into an *rpoB* allelic type (AT); 2 isolates are classified into the same AT if they share identical sequence of the 632-nt *rpoB* fragment analyzed. At least one isolate for a given *rpoB* AT was also characterized by a partial 16S rDNA sequencing. A 616-nt fragment of 16S rDNA was analyzed to aid in genus and species classification of isolates with a given *rpoB* AT. A large *rpoB* sequence database linked to appropriate 16S rDNA-based genus and species data is used to facilitate this characterization method, which has been widely used in the past (Huck et al., 2007a,b; Huck et al., 2008; Ranieri and Boor, 2009; Ranieri et al., 2009; Ivy et al., 2012; Masiello et al., 2014).

For identification, individual colonies of a given isolate were first streaked onto a BHI agar plate, followed by overnight incubation at appropriate temperature (21, 32, 37, or 55°C), to obtain individual colonies. A single colony was transferred into a 0.2-mL PCR tube using a sterile toothpick, followed by lysis by heating in a 1,200-W microwave for 3 min. The obtained lysate was resuspended in 50 μL of nuclease-free water, and 2 μL of a given lysate was used to perform a 25- μL PCR, amplifying either a 740-nt *rpoB* fragment or a 763-nt 16S rDNA fragment. The PCR products were verified for size using gel electrophoresis and purified using the ExoSAP method (Dugan et al., 2002). Bidirectional Sanger sequencing of the PCR product was performed

Table 1. Phenotypic characteristics of isolates included in the isolate set of aerobic dairy spoilers

Cornell ID ¹	Genus and species ²	Growth in skim milk broth at ⁴						β-Gal at ⁵ T (°C), ⁶ 24 h	Proteolytic activity at ⁷			Lipolytic activity at ⁸			Source ⁹		
		rpoB AT ⁻³	6°C, 21 d		21°C, 24 h		32°C, 24 h		37°C, 24 h		55°C, 24 h		6°C, 14 d	21°C, 48 h		T (°C), ⁶ 48 h	
			N	L	H ¹¹	H ¹¹	M		N	N	N	N					N
Gram-positive isolates																	
FSL R5-0215	<i>Bacillus</i> cf. <i>aerophilus</i> ¹⁰	135	N	L	H ¹¹	M	N	– (32°C)	ND	+	+	+	ND	+	+	Pasteurized fluid milk	
FSL H7-0968	<i>Bacillus licheniformis</i>	1	N	L	H ¹¹	M	N	– (32°C)	ND	–	–	–	ND	+	+	Pasteurized fluid milk	
FSL J3-0143	<i>Bacillus licheniformis</i>	1	N	L	M	H ¹¹	N	– (37°C)	ND	– ¹²	++	++	ND	– ¹²	+	Pasteurized fluid milk	
FSL R5-0860	<i>Bacillus</i> sp. ¹³	158	N	H	H ¹¹	H	N	– (32°C)	ND	+	+	+	ND	+	+	Pasteurized fluid milk ¹⁴	
FSL J3-0113	<i>Bacillus cereus</i> s.l. ¹⁵	417	N	H ¹¹	H	M	N	– (21°C)	ND	++	++	++	ND	+	+	Pasteurized fluid milk	
FSL J3-0124	<i>Bacillus weihenstephanensis</i>	3	N	H ¹¹	H	N	ND	– (21°C)	ND	++	++	++	ND	+	+	Pasteurized fluid milk	
FSL H7-0687	<i>Bacillus weihenstephanensis</i>	3	M	H ¹¹	H	N	ND	– (21°C)	+	+	+	+	+	+	+	Pasteurized fluid milk	
FSL J3-0123	<i>Bacillus weihenstephanensis</i>	513	N	M	H ¹¹	N	ND	– (32°C)	ND	++	++	++	ND	+	+	Pasteurized fluid milk	
FSL J3-0137	<i>Bacillus muralis</i>	512	N	M	H ¹¹	M	N	– (32°C)	ND	–	–	–	ND	+	+	Pasteurized fluid milk	
FSL H8-0287	<i>Paenibacillus xylinolyticus</i> , <i>Paenibacillus pabuli</i>	100	M	L	M ¹¹	N	ND	+	(32°C)	–	–	–	++	+	+	Pasteurized fluid milk	
FSL R5-0808	<i>Paenibacillus glucanolyticus</i>	159	L	M ¹¹	L	N	ND	+	(21°C)	–	–	–	+	–	–	Pasteurized fluid milk ¹⁶	
FSL R5-0192	<i>Paenibacillus amylolyticus</i> s.l. ¹⁵	23	N	L	M ¹¹	N	ND	+	(32°C)	ND	–	+	ND	+	+	Pasteurized fluid milk	
FSL H7-0689	<i>Paenibacillus amylolyticus</i> s.l. ¹⁵	23	M	L ¹¹	L	N	ND	+	(21°C)	–	–	–	++	+	+	Pasteurized fluid milk	
FSL J3-0122	<i>Paenibacillus amylolyticus</i> s.l. ¹⁵	23	H	L ¹¹	N	N	ND	+	(21°C)	–	–	–	++	+	+	Pasteurized fluid milk	
FSL H8-0237	<i>Paenibacillus odorifer</i>	15	H	L	L ¹¹	N	ND	+	(21°C)	–	–	–	–	–	–	Pasteurized fluid milk	
FSL J3-0153	<i>Paenibacillus odorifer</i>	46	H	M ¹¹	L	N	ND	+	(21°C)	–	–	–	–	–	–	Pasteurized fluid milk	
FSL J3-0155	<i>Paenibacillus odorifer</i>	35	H	L	M ¹¹	N	ND	+	(32°C)	–	–	–	–	–	–	Pasteurized fluid milk	
FSL J3-0159	<i>Paenibacillus odorifer</i>	7	H	L	M ¹¹	N	ND	+	(32°C)	–	–	–	–	–	–	Pasteurized fluid milk	
FSL R7-0277	<i>Paenibacillus</i> sp. ¹³	45	L	L ¹¹	L	N	ND	+	(21°C)	–	+	+	+	–	–	Pasteurized fluid milk ¹⁷	
FSL R7-0269	<i>Paenibacillus</i> sp. ¹³	163	N	L ¹¹	L	N	ND	+	(21°C)	ND	–	–	ND	–	–	Pasteurized fluid milk ¹⁸	
FSL A5-0031	<i>Paenibacillus</i> sp. ¹³	511	N	N	N	N	ND	+	(32°C) ¹⁹	–	– ¹²	–	++	– ¹²	–	Pasteurized fluid milk ¹⁶	
FSL A5-0030	<i>Paenibacillus peoriae</i>	179	H	M	H	H ¹¹	N	+	(37°C)	+	+	+	–	–	–	Pasteurized fluid milk	
FSL J3-0120	<i>Paenibacillus</i> cf. <i>peoriae</i> ¹⁰	340	H	M	H ¹¹	M	N	+	(32°C)	+	+	++	+	–	–	Pasteurized fluid milk	

Continued

Table 1 (Continued). Phenotypic characteristics of isolates included in the isolate set of aerobic dairy spoilers

Cornell ID ¹	Genus and species ²	Growth in skim milk broth at ⁴						β-Gal at ⁵		Proteolytic activity at ⁷			Lipolytic activity at ⁸			Source ⁹		
		6°C, 21 d	6°C, 21 d	21°C, 24 h	32°C, 24 h	37°C, 24 h	55°C, 24 h	β-Gal at ⁵	6°C, 14 d	21°C, 48 h	T (°C), 48 h	6°C, 14 d	21°C, 48 h	T (°C), 48 h				
		rpoB AT ³						T (°C), 24 h										
FSL H8-0551	<i>Paenibacillus</i> cf. <i>peoriae</i> ¹⁰	157	H	M	H ¹¹	H	N	+	(32°C)	+	+	++	(32°C)	+	−	−	Spore-treated milk ²⁰	
FSL R5-0213	<i>Viridibacillus arenosi</i>	17	H	H	H ¹¹	H	M	ND	−	(21°C)	−	−	(21°C)	+	+	+	Pasteurized fluid milk	
FSL W8-0149	<i>Geobacillus</i> sp. ¹³	357	ND	ND	ND	ND	N	M ¹¹	+	(55°C)	ND	ND	−	(55°C)	ND	ND	++	Dairy-powder production ²¹
FSL W8-0150	<i>Anoxybacillus</i> sp. ¹³	353	ND	ND	ND	N	H ¹¹	M	+	(37°C)	ND	ND	−	(37°C)	ND	ND	+	Dairy-powder production ²¹
FSL W8-0331	<i>Aeribacillus pallidus</i>	376	ND	ND	ND	N	N	N	−	(55°C) ¹⁹	ND	ND	−	(55°C) ^{19,22}	ND	ND	−	Dairy-powder production ²³
FSL W8-0352	<i>Ureibacillus</i> sp. ¹³	468	ND	ND	ND	N	N	N	−	(37°C) ¹⁹	ND	ND	−	(37°C) ^{19,22}	ND	ND	+	Dairy-powder production ²⁰
FSL W6-0449	<i>Lactococcus lactis</i>	NA	H	H	H	H	H ¹¹	N	+	(37°C)	−	−	−	(37°C)	−	−	−	Pasteurized fluid milk ²⁴
FSL R5-0726	<i>Leuconostoc lactis</i>	NA	M	M	M	H ¹¹	H	N	+	(32°C)	−	−	−	(32°C)	−	−	−	Pasteurized fluid milk
FSL W5-0563	<i>Streptococcus thermophilus</i>	NA	N	M	H	H	H ¹¹	N	+	(37°C)	ND	−	−	(37°C)	ND	−	−	Pasteurized fluid milk ²⁵
FSL W6-0423	<i>Enterococcus</i> sp. ²⁶	NA	N	M	M	M	H ¹¹	N	−	(37°C)	ND	−	−	(37°C)	ND	−	−	Pasteurized fluid milk ²⁴
FSL W5-0486	<i>Staphylococcus pasteurii</i>	NA	N	L	H ¹¹	M	N	N	−	(32°C)	ND	−	−	(32°C)	ND	−	−	Pasteurized fluid milk ¹⁶
FSL W5-0353	<i>Micrococcus</i> sp. ²⁶	NA	N	M	M	H ¹¹	M	N	−	(32°C)	ND	+	++	(32°C)	ND	+	++	Pasteurized fluid milk ²⁵
Gram-negative isolates																		
FSL W5-0777	<i>Escherichia fergusonii</i>	NA	N	M	H	H	H ¹¹	N	+	(37°C)	ND	−	−	(37°C)	ND	+	+	Pasteurized fluid milk ²⁵
FSL W4-0242	<i>Enterobacter</i> sp. ²⁶	NA	H	H	H	H ¹¹	H	N	+	(32°C)	−	−	−	(32°C)	−	−	−	Pasteurized fluid milk
FSL W4-0268	<i>Hafnia paralvei</i>	NA	H	M	H ¹¹	H	H	N	+	(32°C)	−	−	−	(32°C)	−	−	−	Pasteurized fluid milk
FSL W4-0276	<i>Citrobacter</i> sp. ²⁶	NA	H	H	H	H ¹¹	H	N	+	(32°C)	−	−	−	(32°C)	−	−	−	Pasteurized fluid milk
FSL P4-0811	<i>Serratia</i> sp. ²⁶	NA	H	H	H	H ¹¹	N	N	+	(32°C)	+	−	−	(32°C)	++	+	+	Pasteurized fluid milk
FSL A6-0213	<i>Rahnella inusitata</i>	NA	H	H	H ¹¹	H	N	N	+	(21°C)	−	−	−	(21°C)	−	−	−	Ropy pasteurized milk
FSL W5-0628	<i>Klebsiella</i> sp. ²⁶	NA	N	H	H	H ¹¹	H	N	+	(32°C)	ND	−	+	(32°C)	ND	−	++	Ropy raw milk
FSL W4-0273	<i>Raoultella</i> sp. ²⁶	NA	H	H	H	H ¹¹	H	N	+	(32°C)	−	−	−	(32°C)	−	−	−	Pasteurized fluid milk
FSL W5-0203	<i>Pseudomonas fluorescens</i>	NA	H	H	H	H ¹¹	N	ND	−	(32°C)	++	++	++	(32°C)	++	++	++	Cheese (pasteurized milk) ²⁷
FSL W7-0098	<i>Pseudomonas fragi</i>	NA	H	M ¹¹	M	M	N	ND	−	(21°C)	++	−	−	(21°C)	++	+	+	Pasteurized fluid milk
FSL R5-0202	<i>Pseudomonas</i> sp. ²⁶	NA	H	M	H ¹¹	N	N	ND	−	(32°C)	++	++	++	(32°C)	++	++	++	Pasteurized fluid milk

Continued

Table 1 (Continued). Phenotypic characteristics of isolates included in the isolate set of aerobic dairy spoilers

Cornell ID ¹	Genus and species ²	Growth in skim milk broth at ⁴						β-Gal at ⁵		Proteolytic activity at ⁷			Lipolytic activity at ⁸			Source ⁹
		rpoB AT ³	6°C,	21°C,	32°C,	37°C,	55°C,	T (°C), ⁶	24 h	6°C,	21°C,	48 h	6°C,	21°C,	48 h	
			21 d	24 h	24 h	24 h	24 h			14 d	48 h	48 h	14 d	48 h	48 h	
FSL R5-0318	<i>Pseudomonas</i> sp. ²⁶	NA	H	H ¹¹	H	N	ND	–	(21°C)	–	–	–	+	+	+	Pasteurized fluid milk
FSL J3-0127	<i>Acinetobacter</i> sp. ²⁶	NA	H	H	H ¹¹	N	ND	–	(32°C)	–	–	–	++	++	++	Pasteurized fluid milk
FSL J3-0142	<i>Brevundimonas</i> sp. ²⁶	NA	H	L ¹¹	N	N	ND	–	(21°C)	ND	–	–	ND	+	+	Pasteurized fluid milk
FSL W6-0482	<i>Flavobacterium</i> sp. ²⁶	NA	H	M ¹¹	N	ND	ND	+	(21°C)	++	+	+	+	+	+	Pasteurized fluid milk ²⁴
FSL A5-0095	<i>Vibrio casei</i> ²⁸	NA	N	L ¹¹	N	N	ND	–	(21°C)	ND	–	–	ND	+	+	Cheese (raw milk) ²⁹

¹Cornell University Food Safety Laboratory (FSL) isolate designation.

²Genus and species identification was based on 16S rDNA or *rpoB* data.

³*rpoB* allelic type. All gram-positive, sporeforming bacteria were typed based on *rpoB*. NA = not applicable.

⁴Relative growth rate in skim milk broth [$\log_{10}(\text{FINAL}) - \log_{10}(\text{INITIAL})$]; N = <1 log cfu/mL (no growth), L = 1–2 log cfu/mL (low growth), M = 2–4 log cfu/mL (medium growth), H = >4 log cfu/mL (high growth), ND = not determined. Result of 2 independent trials. Additional third trial was performed if results of previous 2 were inconsistent (more than 1.0 log cfu/mL apart).

⁵β-galactosidase activity, hydrolysis of bromo-chloroindolyl-galactopyranoside: – = no activity, negative reaction, + = activity present, positive reaction.

⁶Temperature of highest 24-h growth rate in skim milk broth (see footnote 11).

⁷Proteolytic activity, hydrolysis of milk proteins (skim milk agar): – = no activity, negative reaction; + = weak activity, weak reaction; ++ = strong activity, strong reaction.

⁸Lipolytic activity, hydrolysis of lipase reagent (spirit blue agar): – = no activity, negative reaction; + = weak activity, weak reaction; ++ = strong activity, strong reaction.

⁹If not stated otherwise, the isolates were obtained from commercially pasteurized fluid milk (HTST).

¹⁰cf. = confer. Isolate matching 16S rDNA sequence (i.e., 97.0–98.0% sequence similarity) of more than one type strain from family *Bacillaceae*.

¹¹Temperature at which highest growth rate was detected after 24 h in skim milk broth.

¹²Growth on agar plate was poor.

¹³Isolate not matching 16S rDNA sequence (i.e., <98.0% sequence similarity) of any type strain from family *Bacillaceae*.

¹⁴Pilot plant pasteurized milk (80°C/25 s).

¹⁵s.l. = sensu lato. Isolate matching 16S rDNA sequence (i.e., >98.0% sequence similarity) of more than one type strain from family *Bacillaceae*.

¹⁶Pilot plant pasteurized milk (77°C/25 s).

¹⁷Pasteurized skim chocolate milk with a sensory defect.

¹⁸Pilot plant pasteurized milk (82.5°C/25 s).

¹⁹Temperature of highest 24-h growth rate in brain heart infusion broth.

²⁰Spore-treated raw milk (80°C/12 min).

²¹Skim milk, heat treated for highly heat-resistant spore count (100°C/30 min).

²²No growth on agar plate.

²³Evaporated milk, heat treated for highly heat-resistant spore count (100°C/30 min).

²⁴Laboratory pasteurized milk (63°C/30 min).

²⁵Pilot plant pasteurized milk (72°C/25 s).

²⁶Isolate not matching 16S rDNA sequence (i.e., <99.9% sequence similarity) of any type strain. See footnote 28 for exception.

²⁷Latin-style cheese (fresh, low acid).

²⁸Isolate matching 16S rDNA sequence (97.0% sequence similarity) of type strain of *Vibrio casei*.

²⁹Washed rind cheese.

by the Life Sciences Core Laboratory Center (Cornell University).

Determination of Isolate Growth Characteristics in Skim Milk Broth at Different Temperatures

To assess growth in skim milk broth (SMB; BD, Franklin Lakes, NJ) at different temperatures, all isolates were first plated on BHI agar. Following overnight incubation at the appropriate temperature (21, 32, 37, or 55°C), a single colony was inoculated into 5 mL of BHI broth and incubated aerobically at the same temperature used to initially grow an isolate on BHI agar. After 18 to 24 h of incubation (plating showed that bacterial numbers ranged between 1.1×10^5 and 1.7×10^9 cfu/mL), 1 mL of culture was pelleted (13,000 rpm/1 min), and the cell pellet was resuspended in 1 mL of phosphate buffer. The resuspended cell pellet was serially diluted appropriately (to obtain an approximate cell concentration of 10^3 cfu/mL) and 1 mL of it was used to inoculate 9 mL of sterile SMB. The initial concentration at inoculation was between 10^2 and 10^3 cfu/mL. The inoculated SMB was incubated at 21, 32, 37, or 55°C for 24 h, and bacterial numbers were enumerated by spiral-plating appropriate dilutions on BHI agar (Difco, BD) using Autoplate 4000 (Spiral Biotech, Norwood, MA). Bacterial colonies were counted after 24 to 48 h (depending on colony growth morphology) using a Q-count (Spiral Biotech). When assessing the cold growth, the inoculated SMB was held for 21 d at 6°C ($\pm 0.5^\circ\text{C}$), and the bacterial numbers were evaluated after 14 and 21 d. All growth tests were performed in 2 repetitions; if the bacterial numbers of 2 repetitions were not within 1 log, a third growth test was performed. When assessing cold growth at 6°C, a third growth test was performed if bacterial numbers of the 2 repetitions were not within 1 log at either 14 or 21 d of incubation. The growth data were used to calculate the growth rate by subtracting the initial-day concentration from the final-day concentration [$\log_{\text{day(FINAL)}} - \log_{\text{day(INITIAL)}}$].

Determination of Enzymatic Activity

To assess enzymatic activities (i.e., lipolytic, proteolytic, and β -galactosidase activity), isolates were first plated on BHI agar and incubated overnight at 21, 32, 37, or 55°C. A single colony from an overnight plate was subsequently streaked in duplicate on different agar plate media as detailed below to assess specific enzymatic activity. Each type of enzymatic activity was determined at the temperature at which individual isolates demonstrated the most rapid growth in SMB.

Proteolytic and lipolytic activity were additionally determined at 6 and 21°C.

β -Galactosidase Activity. A colony was streaked onto 2 BHI agar plates, one with and one without an overlay of 100 μL of a 40- $\mu\text{g/mL}$ solution of bromo-chloroindolyl-galactopyranoside (Gold Biotechnology, St. Louis, MO), followed by incubation at appropriate temperature (21, 32, 37, and 55°C) for 24 h. Blue colonies on the plates containing bromo-chloroindolyl-galactopyranoside were indicative of β -galactosidase activity.

Proteolytic Activity. A colony was streaked on skim milk agar (SMA; Difco). Plates were incubated at appropriate temperature (21, 32, 37, and 55°C) and analyzed after 48 h of incubation. When proteolytic activity was assessed at 6°C, the plates were analyzed after 14 d of incubation. Each plate was scored on a 3-point scale for proteolytic activity: (–) was given to plates with no visible zone of clearing around the growth, (+) was given to plates with weak zone of clearing (with no defined edge) or to plates with zone of clearing (with defined edge) along only a part of the growth, and (++) was given to plates with zone of clearing (with defined edge) around the entire length of the growth.

Lipolytic Activity. A colony was streaked on Spirit Blue agar (Difco) containing an emulsion of pre-prepared lipase reagent containing a mixture of Tributyrin and Polysorbate 80 (Difco). Plates were incubated at appropriate temperature (21, 32, 37, and 55°C) and analyzed after 48 h of incubation. When lipolytic activity was assessed at 6°C, the plates were analyzed after 14 d of incubation. Each plate was scored on a 3-point scale for lipolytic activity: (–) was given to plates with no visible zone of clearing, (+) was given to plates with weak (less than 2 mm) zone of clearing, and (++) was given to plates with strong (more than 2 mm) zone of clearing around the bacterial growth.

Internet-Based Access

Data on all isolates presented in the isolate set of dairy spoilers, including source information, date of isolation, and partial 16S rDNA and *rpoB* sequences, can be found in the publicly available FoodMicrobeTracker database (<http://www.foodmicrobetracker.com>). FoodMicrobeTracker can be searched for individual isolates by use of the appropriate FSL ID (e.g., FSL A5–0030; see Table 1) to access information (Vangay et al., 2013).

Isolate Availability

The isolate set of dairy spoilers is maintained by the FSL. Request for isolates or more information should

be addressed to the corresponding author of this article (M. Wiedmann).

RESULTS

Major Groups and Associated Isolate Diversity

Isolates included in this standard bacterial set of aerobic dairy spoilers are listed in Table 1 and are separated into 2 main groups, gram-positive and gram-negative bacteria; the gram-positive bacteria can be further separated based on their capability to form endospores. Different sporeforming, gram-positive bacteria represent a large part of this bacterial set, as these types of organisms represent a major challenge in both fluid milk spoilage and dairy-powder quality. Because the taxonomy of sporeforming, aerobic bacteria of the family *Bacillaceae* is in considerable flux with frequent reclassification and description of new species and genera, it is sometimes difficult to assign a species name to an isolate; selection of isolates for inclusion in the isolate set described here thus used *rpoB* AT, which allow for reliable classification into different genotypes. In a few cases, a given *rpoB* AT is represented in the set by 2 or 3 different isolates, for example if isolates with a given *rpoB* AT represented distinct phenotypic characteristics. In our isolate set, the genus *Bacillus* is represented by 9 isolates classified into 7 different *rpoB* AT, whereas the genus *Paenibacillus* is represented by 15 isolates classified into 13 different *rpoB* AT. All 9 *Bacillus* isolates and 14 *Paenibacillus* isolates were obtained from samples of fluid milk by standard plate-counting techniques. One *Paenibacillus* isolate (FSL H8-0551) and one *Ureibacillus* isolate (FSL W8-0352) were isolated from spore-treated raw milk (80°C/12 min). The remaining 3 sporeforming isolates (FSL W8-0149, W8-0150, W8-0331), obtained from various points of dairy-powder production, were isolated after heat treatment to select for highly heat-resistant spores (100°C/30 min). The nonsporeforming bacteria represented in this isolate set are lactic acid bacteria and other cocci that were obtained from either commercially pasteurized or pilot plant pasteurized milk or milk pasteurized using the “lab pasteurization” approach (63°C/30 min; Frank and Yousef, 2004).

Among the 16 gram-negative isolates included in the isolate set reported here, 7 represent coliforms and 4 represent *Pseudomonas* species commonly linked to PPC. Among the *Pseudomonas* isolates, isolate FSL W5-0203 is unique because it was the cause of blue color defect in cheese due to PPC (Martin et al., 2011a). Additionally, one isolate of each *Acinetobacter*, *Brevundimonas*, and *Flavobacterium* are included in the set to represent less common genera involved in PPC. Two

coliform isolates, classified as *Rahnella inusitata* (FSL A6-0213) and *Klebsiella* sp. (FSL W5-0628), represent bacteria linked to the ropy milk defect; these isolates were obtained from ropy samples of pasteurized and raw milk, respectively. The isolate set also includes an isolate of *Vibrio casei*. This recently identified species was shown to be commonly identified in raw-milk cheese samples (Bleicher et al., 2010; Wolfe et al., 2014).

Growth Characteristics

An important factor in the ability of an isolate to cause spoilage of food products is its ability to grow and proliferate under the conditions found in the food product (e.g., pH, water activity, and so on). We have tested all 51 isolates included in the isolates set described here for growth in SMB (see Table 1). Only one *Bacillus* isolate, *B. weihenstephanensis* FSL H7-0687, was able to grow at 6°C. Whereas 8 *Paenibacillus* isolates and one *Viridibacillus* isolate (FSL R5-0213) exhibited high growth rates (>4 log cfu/mL) at 6°C, 3 *Paenibacillus* isolates (FSL R7-0269, A5-0031, R5-0192) were unable to grow at 6°C. The 4 thermotolerant isolates (FSL W8-0149, W8-0150, W8-0331, W8-0352) were not tested for cold growth. Of 6 nonsporeforming, gram-positive bacteria, only *Leuconostoc* (FSL R5-0726) and *Lactococcus* (FSL W6-0449) isolates grew at 6°C. Within the gram-negative group, 3 isolates (FSL W5-0628, W5-0777, A5-0095) were not able to grow in SMB at 6°C; the 13 other gram-negative isolates were able to grow by more than 4 log units at this temperature. These isolates are represented by the genus *Pseudomonas* (FSL W5-0203, W7-0098, R5-0202, R5-0318), coliform bacteria (FSL W4-0268, W4-0276, W4-0242, P4-0811, A6-0213, W4-0273), *Acinetobacter* sp. (FSL J3-0127), *Brevundimonas* sp. (FSL J3-0142), and *Flavobacterium* sp. (FSL W6-0482). The phenotype observed at 6°C identified the *Rahnella* isolate FSL A6-0213 as a strong producer of mucoid substances, consistent with its ability to cause a ropy defect in milk.

Two thermotolerant isolates, *Aeribacillus* sp. FSL W8-0331 and *Ureibacillus* sp. FSL W8-0352, as well as the *Paenibacillus* sp. isolate FSL A5-0031, were unable to grow in SMB at any tested temperature. Whereas most of the *Paenibacillus* isolates (n = 12) were not able to grow at temperatures above 32°C, 3 *Paenibacillus* isolates were able to grow at 37°C, including the isolate *Paenibacillus peoriae* FSL A5-0030, which showed its highest growth rate at this temperature. The thermotolerant isolate *Anoxybacillus* sp. FSL W8-0150 had similar growth rates at both 37 and 55°C, whereas the *Geobacillus* isolate FSL W8-0149 grew only at 55°C. Most of the isolates (n = 6) within the *Bacillus* group were able to grow at 37°C, except for 3 *B. weihenstepha-*

nensis isolates. All 6 nonsporeforming, gram-positive isolates were able to grow at 37°C, whereas none of the *Pseudomonas* isolates grew at this temperature. The psychrotolerant isolates *Rahnella inusitata* FSL A6-0213 and *Serratia* sp. FSL P4-0811 were also unable to grow at 37°C, whereas all other coliform isolates grew well at this temperature (with growth rates above 4 log cfu/mL). *Rahnella inusitata* FSL A6-0213 exhibited poor production of mucoid substances at temperatures above 6°C, whereas the *Klebsiella* isolate FSL W5-0628 produced these substances at 21, 32, and 37°C. Most of the isolates (n = 42) included in the isolate set were able to grow at temperatures 21 and 32°C. For the *Vibrio casei* isolate FSL A5-0095, 21°C was the only tested temperature that allowed some growth within 24 h.

β-Galactosidase, Proteolytic, and Lipolytic Activity

All 9 *Bacillus* isolates as well as *Viridibacillus arenosi* FSL R5-0213, *Aeribacillus* sp. FSL W8-0331 and *Ureibacillus* sp. W8-0352 were negative for β-galactosidase activity (Table 1). The other 2 thermotolerant isolates, *Geobacillus* sp. FSL W8-0149 and *Anoxybacillus* sp. FSL W8-0150, as well as all 15 *Paenibacillus* isolates were positive for β-galactosidase. Lactic acid bacteria (FSL R5-0726, W5-0563, W6-0449) were the only nonsporeforming, gram-positive bacteria that were positive for β-galactosidase. Among the gram-negative bacteria, all 8 coliform isolates as well as one noncoliform (*Flavobacterium* sp. FSL W6-0482) isolate were positive for β-galactosidase activity. All 4 *Pseudomonas* isolates as well as 3 other noncoliform isolates (FSL J3-0127, J3-0142, A5-0095) were negative for β-galactosidase activity.

Seven *Bacillus* isolates were positive for proteolytic activity on SMA. The 2 *Bacillus* isolates negative for proteolytic activity were *B. licheniformis* FSL H7-0968 and *B. muralis* FSL J3-0137. The thermotolerant isolates *Aeribacillus pallidus* FSL W8-0331 and *Ureibacillus* sp. FSL W8-0352 were unable to grow on SMA. The thermotolerant isolates *Geobacillus* sp. FSL W8-0149 and *Anoxybacillus* sp. FSL W8-0150, which did grow on SMA, exhibited no proteolytic activity. All 3 *P. peoriae*-group isolates as well as *Paenibacillus amylolyticus* s.l. FSL R5-0192 and *Paenibacillus* isolate FSL R7-0277 tested positive for proteolytic activity. The other 10 *Paenibacillus* isolates, including all 4 *P. odorifer* isolates, were negative for proteolytic activity. Among the nonsporeforming, gram-positive bacteria, only *Micrococcus* sp. FSL W5-0353 showed proteolytic activity. Two *Pseudomonas* isolates (FSL W5-0203, R5-0202) had a strong proteolytic activity at all tested temperatures. Isolate *Pseudomonas fragi* (FSL W7-0098) was positive for proteolytic activity only when

incubated at 6°C for 14 d, whereas one *Pseudomonas* isolate (FSL R5-0318) tested negative for proteolytic activity at all tested temperatures. *Flavobacterium* sp. FSL W6-0482 exhibited proteolytic activity at 6 and 21°C. *Klebsiella* sp. isolate FSL W5-0628 and *Serratia* sp. isolate FSL P4-0811 were the only 2 coliforms with detected proteolytic activity.

All 9 *Bacillus* isolates, all 3 *P. amylolyticus* s.l. isolates, as well as the *Paenibacillus xylanilyticus* and *Paenibacillus pabuli* isolate FSL H8-0287 and *V. arenosi* FSL R5-0213 were positive for lipolytic activity at all tested temperatures that supported growth. Five *Paenibacillus* isolates (FSL R5-0808, R7-0277, A5-0031, J3-0120, H8-0551) were positive for lipolytic activity only at 6°C. All 4 *P. odorifer* isolates, as well as 2 other *Paenibacillus* isolates (FSL R7-0269, A5-0030), tested negative for lipolytic activity. *Aeribacillus pallidus* (FSL W8-0331) is the only thermotolerant isolate negative for lipolytic activity. Within the group of nonsporeforming, gram-positive bacteria, *Micrococcus* sp. (FSL W5-0353) is the only isolate positive for lipolytic activity. Two nonpsychrotolerant coliform isolates, *Escherichia fergusonii* (FSL W5-0777) and *Klebsiella* sp. (FSL W5-0628), were positive for lipolytic activity, whereas *Serratia* sp. FSL P4-0811 was the only psychrotolerant coliform isolate positive for lipolytic activity. All 8 noncoliform, gram-negative isolates, including all 4 *Pseudomonas* isolates, were positive for lipolytic activity at all tested temperatures.

DISCUSSION

Publicly available isolate sets are a well-established way of providing relevant microbial isolates for research and development. Although many microbial sets are established and available for foodborne pathogens [e.g., *Salmonella* (Boyd et al., 1993), *E. coli* (Ochman and Selander, 1984)], no current isolate sets exist that focus on industry-specific organisms responsible for food spoilage. This manuscript reports the establishment of one such isolate set, which consists of various isolates responsible for spoilage of milk and dairy products.

Taxonomic, Phenotypic, and Genotypic Diversity of Isolate Set in Relation to Known Dairy-Spoilage Organisms

This Standard Bacterial Set for Research on Current Dairy Spoilage consists of 51 individual isolates, including 35 gram-positive and 16 gram-negative isolates. Because fluid milk represents the most perishable dairy product, most of the isolates represented in this set originate from pasteurized milk, with additional isolates from pasteurized chocolate milk and raw milk.

The bacterial set also includes 4 relevant isolates originating from dairy-powder production and 2 isolates from cheese.

Gram-positive isolates include several nonsporeforming (e.g., *Staphylococcus* and lactic acid bacteria) as well as a large number of sporeforming bacteria. Sporeforming bacteria are represented predominately by genera *Bacillus* and *Paenibacillus*, which have previously been shown to be important dairy-spoilage organisms (Huck et al., 2007a; Ledenbach and Marshall, 2009; Ranieri and Boor, 2009; Ranieri et al., 2012). From our previous study (Ivy et al., 2012) we included isolates with *rpoB* AT representing the 3 most prominent dairy-associated *Bacillus* clades, one isolate representing a less common clade (*B. muralis*), and also a *Viridibacillus* isolate as a representative of the fourth prominent clade. The strains were also selected to represent *rpoB* AT of the 5 most prominent *Paenibacillus* clades (V, VII, VIII, XI, X) associated with dairy. For some clades or *rpoB* AT, we selected multiple isolates to represent phenotypic diversity. For example, our isolate set includes 3 *B. weihenstephanensis* isolates, including 2 with *rpoB* AT3; these isolates exhibit different proteolytic and cold growth capabilities. Another example are the 3 *P. amylolyticus* s.l. *rpoB* AT23 isolates, which are exhibiting none, medium, and high level of growth at 6°C; the isolate unable to grow at cold temperature is also the only of 3 isolates that exhibits any proteolytic activity on SMA.

Our isolate set also includes gram-positive sporeforming bacteria that originate from production of dairy powder. These isolates were obtained from samples collected at various points of dairy-powder production (Watterson et al., 2014) and represent both standard thermophilic bacteria (*Ureibacillus* sp.) that produce spores able to survive heat treatment of 80°C/12 min, as well as so-called highly heat-resistant thermophilic bacteria (*Geobacillus* sp., *Anoxybacillus* sp., and *Aeribacillus pallidus*) that produce spores able to survive heat treatment at 100°C/30 min. All 4 genera were previously reported as contaminants of dairy powder and dairy-powder production facilities (Rückert et al., 2004; Burgess et al., 2010; Lücking et al., 2013; Watterson et al., 2014). Isolates representing the genus *Anoxybacillus* were identified as the most prevalent bacterial contaminants in dairy powders and a common problem in dairy-powder production regardless of geographic location (Rückert et al., 2004). Different species of the genus *Geobacillus* are also frequently identified as contaminants of dairy powders and inhabitants of dairy-powder production facilities (Ronimus et al., 2003). *Aeribacillus pallidus* (formerly known as *Geobacillus pallidus*; Miñana-Galbés et al., 2010) has also been reported as a dominant species, in dairy powders, that

produces highly thermoresistant spores (Lücking et al., 2013). These bacterial contaminants are generally regarded as hygiene indicators, and only a small part of them can cause actual product defect. The biggest factor in being able to cause product defect is the ability to produce different hydrolytic enzymes (Burgess et al., 2010). Our isolate set includes isolates with and without β -galactosidase activity, as well as isolates with various degrees of lipolytic activity.

Although nonsporeforming, gram-positive bacteria are less common as dairy-spoilage organisms, they are well documented contaminants of raw milk and the dairy-processing environment (Cleto et al., 2012). This group of bacteria can cause PPC and spoilage of fluid milk and other dairy products (Ledenbach and Marshall, 2009; Martin et al., 2011b). Our isolate set includes an isolate *Leuconostoc lactis* from a commercially pasteurized milk (Ranieri and Boor, 2009), as well as several isolates obtained from laboratory pasteurization of raw milk. Heat treatments of milk samples from which these isolates were obtained include 63°C/30 min (*Lactococcus lactis*, *Enterococcus* sp.), 72°C/25 s (*Micrococcus* sp., *Streptococcus thermophilus*), and 77°C/25 s (*Staphylococcus pasteurii*; Caplan et al., 2013).

Gram-negative isolates included in the isolate set represent key spoilage organisms, previously identified as important in dairy products (Dogan and Boor, 2003; Hantsis-Zacharov and Halpern, 2007; Ledenbach and Marshall, 2009; Martin et al., 2012a), including *Pseudomonas*, *Acinetobacter*, *Brevundimonas*, *Flavobacterium*, and various coliform bacteria. Four *Pseudomonas* isolates were included in our set to represent genotypic and phenotypic as well as isolation source diversity. One *Pseudomonas fluorescens* isolate was obtained from a cheese sample with a blue color defect (Martin et al., 2011a), and the other 3 are fluid milk isolates (Ranieri and Boor, 2009), which were also previously used by our group to evaluate various selective media for detection of *Pseudomonas* species (Van Tassel et al., 2012).

In another study from our group, on milk contamination with psychrotolerant coliform bacteria, 5 genera (*Enterobacter*, *Hafnia*, *Citrobacter*, *Serratia*, and *Raoultella*) were identified as the most prevalent (S. N. Masiello, Cornell University, Ithaca, NY, personal communication). Representatives of all 5 prevalent psychrotolerant coliform genera are included in this isolate set. The genus *Enterobacter* was the most prevalent genus in fluid milk, and the specific *Enterobacter* isolate (FSL W4-0242) included in the isolate set represents the most prevalent 16S rDNA sequence type among all psychrotolerant coliforms isolated from different fluid-milk samples. We also included a representative of a less prevalent psychrotolerant genus, *Rahnella*. This isolate was classified as *Rahnella inusitata* (formerly

known as Genomspecies 3 of genus *Rahnella*; Brady et al., 2014) and was obtained from pasteurized milk exhibiting a ropy defect. Additionally, we included a *Klebsiella* sp. isolate, which was obtained from a raw-milk sample that showed a ropy defect. The genus *Klebsiella* has previously been identified as the causative organism of ropy milk (Cheung and Westhoff, 1983). Although the *Klebsiella* sp. ropy milk isolate is able to produce mucoid substances and cause ropiness, it is not capable of growing at refrigeration temperatures; the *Rahnella inusitata* isolate is capable of cold growth and will cause a substantial ropy defect within 21 d of incubation at 6°C. Although rare (Mader, 1940; Cheung and Westhoff, 1983), the ropy defect in milk is severe and will require additional research to characterize the causative organisms and develop strategies to eliminate this type of contamination. In addition to these coliform isolates discussed above, we also included in our isolate set an isolate of *Escherichia fergusonii*. Based on DNA-DNA hybridization (60% similarity), *E. fergusonii* is the closest relative of *E. coli* (Lawrence et al., 1991) and most likely often times interpreted as *E. coli* when using classical counting and identification methods. In an on-going study by our group (A. Trmčić, unpublished data), *E. fergusonii* was identified as the most prevalent coliform in raw and pasteurized cheese; hence, inclusion of this species in this isolate set was deemed appropriate.

We characterized the isolates included in this isolate set by determining growth in SMB, proteolytic and lipolytic activity at different temperatures, as well as presence of β -galactosidase. This basic characterization provides important initial information that will allow others to select isolates for further characterization. One of the future steps in characterization should be to fully identify and classify isolates included in this isolate set by full genome sequencing. Full genome sequencing and other molecular biology tools can also help identify the genomic features responsible for spoilage potential of these isolates. Nine gram-positive sporeforming isolates featured in this isolate set were already included in a recent comparative full-genome study performed by our group (Moreno Switt et al., 2014). The results of this study identified several different genomic features involved in the ability of strains to grow and spoil refrigerated fluid milk but also opened some questions that need to be addressed in the future. Upcoming work will include a comparative full-genome study on bacteria that cause ropy milk defect (A. Trmčić, unpublished data), using 2 of the isolates included in the isolates set described here.

Although this isolate set does represent the key aerobic spoilage bacteria associated with the dairy environment, other aerobic as well as anaerobic spoilage

bacteria (e.g., *C. tyrobutyricum*) still need to be added to comprehensively capture the entire range of dairy-associated spoilage microorganisms. Furthermore, yeasts and molds are becoming increasingly important as spoilage organisms, and they too need to be eventually included in the dairy-spoilage isolate set.

Potential Applications of the Isolate Set

The isolates included in this standard set of dairy-spoilage bacteria can be used in studies dealing with different aspects of spoilage characterization, detection, and prevention. One use of this set should be to obtain basic knowledge on the adaptation and survival of spoilage organisms under different dairy-relevant conditions (Huck et al., 2007a; Masiello et al., 2014). Use of these isolates to further study mechanisms involved in microbial spoilage of dairy products (e.g., identification of specific enzymes responsible for defects such as ropy milk) would not only provide relevant basic scientific information, but could also help to develop novel strategies to prevent and minimize dairy spoilage. Genomic studies on individual spoilage organisms already proved to be useful in this regard (Moreno Switt et al., 2014), but these studies must also be supported by phenotypic or even epigenetic studies.

The standard set of dairy-spoilage bacteria reported here will also be valuable for the evaluation of established and the development of new detection methods as well as control and prevention strategies. Many of the methods used for evaluation of dairy-product quality have been in use for decades, despite the vast improvement in dairy and milk quality (Boor, 2001; Martin et al., 2012a), identification and possibly emergence of new spoilage organisms (Ranieri and Boor, 2009; Ivy et al., 2012), and technological and processing advances (Martín et al., 2010; Ranieri et al., 2012; Masiello et al., 2014). Thus, the need exists to validate and develop detection and control methods for spoilage organisms, which requires a relevant isolate set that represents the current organisms of interest and relevance to the dairy industry. For example, the isolates described here could be used as target organisms in controlled challenge tests and are likely to represent the range of behavior of spoilage organisms in a real production process.

CONCLUSIONS

Although this isolate set represents a wide range of the bacterial dairy-spoilage organisms known today, further development and expansion of this isolate set will be necessary to capture the diversity and dynamic nature of microbial spoilage. Importantly, this isolate set does not yet include anaerobic spoilage organisms

or spoilage yeast and molds. This isolate set, together with data curation in FoodMicrobeTracker (<http://www.foodmicrobetracker.com>), already provides a unique resource though that will help advance knowledge of dairy-spoilage organisms as well as aid industry in developing new control strategies. Isolates included in this set have already been used by our group in several different research studies (Ranieri et al., 2009; Martin et al., 2011a, 2012a,b; Ivy et al., 2012; Van Tassel et al., 2012; Moreno Switt et al., 2014) and have also been distributed to other research groups and industry partners.

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