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Psychrotrophic Clostridia Causing Spoilage in Cooked Meat and Poultry Products

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ABSTRACT

Certain types of commercially produced noncured turkey breast and roast beef are precooked in situ, stored at 4°C or below, and typically given use by dates of greater than 50 days. While of rare, sporadic occurrence, an unpleasant spoilage characterized by strong H_2S odor and gas production has been observed in these products. This spoilage is due to the growth of psychrotrophic anaerobic sporeformers. Isolates from roast beef resemble *Clostridium laramie* while isolates from uncured turkey have been designated *C. ctm* for cooked turkey meat. The turkey breast isolates were characterized by temperature growth ranges, carbohydrate fermentations, and other biochemical reactions. Growth of all isolates was inhibited in broth media by 3.0% NaCl, 100 ppm nitrite, 2.0% sodium lactate, or 0.2% sodium diacetate. Inoculated studies were performed with three isolates in cooked turkey product. All three isolates grew and spoiled product at 10 and 3.3°C, and one isolate grew at 0.5 and −3°C. Some differences in growth were observed with the lactate and diacetate treatments in turkey meat among the three isolates. One isolate appeared to utilize the lactate, two were inhibited. Overall, 0.1% diacetate consistently delayed growth, although to different degrees, for all isolates.

There has been increased consumer demand over the past 20 years for products that contain little or no preservatives *(2).* One technology being used to produce these types of products is referred to as sous vide processing. Sous vide, meaning under vacuum *(2, 11),* involves vacuum packaging food in heat-resistant O_2-impermeable barrier bags *(14),* heat processing, and storing at refrigeration temperature to provide an extended shelf life *(2, 3).* Some of the advantages of sous vide processing include a reduced risk of postprocess contamination, increased shelf-life, and improved product quality *(2).* These products have been reported to retain their original aroma, flavor, and texture better than conventionally cooked foods.

The microorganisms of concern in sous vide products are psychrotrophic anaerobic sporeformers, due to the anaerobic environment, heat treatments that are likely to destroy vegetative cells, and storage at refrigeration temperatures *(2, 3).*

Psychrotrophic clostridia have been implicated in the spoilage of both raw and cooked vacuum-packaged refrigerated beef *(7–9, 14, 16, 17),* pasteurized crabmeat *(20),* vacuum-packaged cooked pork *(19),* and vacuum-packed lamb *(6, 7).* Kalchayanand et al. *(17)* and Dainty et al. *(9)* both describe a similar clostridia isolated from vacuum-packaged refrigerated beef. The spoilage was characterized as extensive gas production and foul H_2S odor. Microscopic evaluation revealed large, thick gram-positive rods whose characteristics did not agree with previously recognized *Clostridium* species; thus, each group designated their isolate as a new species, *C. laramie (16)* and *C. estertheticum (8).* Because both groups were investigating the same spoilage incident they are likely one species. The nontoxigenic psychrotrophic anaerobic sporeformer isolated from spoiled cans of pasteurized crabmeat resembled *C. arcticum* but had some distinct differences that pointed to an unrecognized species *(20).* The anaerobic spore-forming rod from vacuum-packaged cooked pork was proposed as a new species, *C. algidicarnis (19).*

Spoilage of vacuum-packaged cooked roast beef and turkey due to clostridial growth occurs very infrequently at refrigeration temperatures. During these events there is no history of temperature abuse. Typically, spoiled beef packages have been extensively swollen, if not blown open, and emit a strong foul H_2S odor. Procedures commonly employed for investigating the spoilage of meat and poultry product are unproductive. Subsequent analysis using anaerobic incubation of sheep blood agar plates and fluid thioglycollate broth sealed with Amojell (Amoco Petroleum Products, Chicago, Ill.), however, did yield growth at 10°C after 2 weeks incubation, thus allowing for isolation of the spoilage organisms. The isolates from roast beef all share the characteristics of *C. laramie* as described by Kalchayanand et al. *(16).*

Spoilage of cook-in-bag noncured turkey breast also has been caused by a psychrotrophic anaerobic sporeformer but the isolates have differed from those recovered from roast beef. In addition, the spoiled turkey products appear normal (i.e., no swelling) until opened when a strong offensive H_2S odor is detected and when cut, the inside of the product has the pinkness of a strawberry milk shake. The isolates from the turkey product have been smaller in size than *C. laramie* and have a wider growth range. The purpose of this study is to provide information on some of

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the isolates recovered from vacuum-packaged roast beef and cook-in-bag turkey breast and to provide possible control measures for their growth.

MATERIALS AND METHODS

Isolation and growth characteristics. Spoiled cook-in-bag roast beef (purge) and turkey breast (core) were analyzed using serial dilutions in presteamed fluid thioglycollate broth (Difco Laboratories, Detroit, Mich.), sealed with Amojell, and incubated at 10°C. When growth was apparent, the isolates were streaked on tryptic soy agar plates containing 5% sheep blood (Micro Diagnostics, Lombard, Ill.) and incubated in GasPak anaerobic jars (BBL, Becton Dickinson, Cockeysville, Md.) at 10°C for a minimum of 14 days. When growth was visible on plates, colonial morphology, hemolytic reaction, Gram reaction, and cell morphology were recorded. Isolated colonies were then transferred to fluid thioglycollate broth for use as inocula to study growth characteristics.

Temperature growth ranges of isolates were determined by inoculating tryptone peptone glucose yeast extract (TPGY) broth *(13, 18)* in triplicate and incubating at 42, 35, 30, 20, 10, and 3.3°C with daily examination over 28 days. Samples that did not show signs of growth, determined by turbidity and gas production, were recorded as negative. By recording the time for growth to become apparent, the optimum growth temperature could be determined. Carbohydrate fermentations and other biochemical tests included hemolysin, lipase, and lecithinase production; gelatin and starch hydrolysis; nitrate reduction; and H_2S production. These analyses were conducted as described by Holdeman et al. *(15)* and Summanen et al. *(23).*

Identification. Six isolates from spoiled turkey breast products were sent for identification to Anatec, Inc. (Blacksburg, Va.), a laboratory specializing in anaerobe identification. The isolates were reported to be *C. ctm,* an unnamed *Clostridium* species isolated from cooked turkey meat. Identification of the isolates was determined by the Microbial Identification System (Microbial ID, Inc., Newark, Del.). This system identifies bacteria by comparing cellular fatty acid profiles with cellular fatty acid profiles from identification libraries.

Toxicity. Isolates (982-92, 1916-92, 3280-94, and 998-95) from four different episodes of spoiled turkey breast were tested for toxin production. Strain 982-92 was obtained from product produced at a different facility than the other three. Isolates were grown anaerobically in TPGY and TPGY with trypsin (TPGYT) at 35°C for 72 h. The trypsinized and nontrypsinized cultures were each injected into two mice intraperitoneally using 0.5 ml of undiluted culture. A portion of each culture was heated at 100°C for 10 min, cooled, and injected into two additional mice to serve as controls *(13).* The mice were observed over a 72-h period.

Sporulation and heat resistance. An isolate of *C. laramie* recovered from spoiled roast beef, sporulated well in fluid thioglycollate broth sealed with Amojell, and incubated at 10°C for 2 weeks. Difficulty, however, was experienced in generating spores from the isolates recovered from turkey. Several different media (cooked meat medium [Difco] *(15, 18, 22),* TPGY broth *(18),* egg meat medium [Difco] *(15, 20, 22, 23)* and tryptone-peptone [TP] broth *(1)* consisting of 5% tryptone [Difco], 0.5% peptone [Difco], 0.05% sodium thioglycollate [Difco], pH 7.2) were tested at 20 and 30°C. Cooked meat medium was shown to produce adequate spores when incubated at 20°C for 7 days. Spores for determining heat resistance were prepared by the method of Anellis et al. *(1).* The spore crops were harvested by centrifugation at 12,000 × *g* for 20 min at 3°C, resuspended in sterile deionized water, and centrifuged again. Spores were resuspended in 20 ml deionized water and stored in cryogenic vials (Nalgene, Rochester, N.Y.) at −20°C. This differs from the referenced method in which suspensions are stored in 100 ml of water over glass beads at 2 to 5°C, conditions that might permit germination of the spores.

Spore heat resistance was determined in phosphate buffer, pH 7.0, using the flask method of Segner *(20).* A 500-ml three-necked round-bottom flask was used for this study. The larger center opening was plugged with a rubber stopper containing a vent tube covered with a fermentation tube. One of the smaller side neck openings was plugged with a solid rubber stopper and used as a sampling port. The second side neck opening was plugged with a solid rubber stopper through which a thermocouple was inserted for recording temperature of the buffer inside the flask. The stoppered flask containing a stirring bar was autoclaved for 15 min at 121°C. An aliquot of 100 ml phosphate buffer was added to the flask that was then placed on a submersible air-operated magnetic stirrer (Cole-Parmer, Niles, Ill.) in a constant temperature water bath (Precision Scientific, Chicago, Ill.). The purpose of the submersible stirrer and stir bar in the flask was to assure continual mixing of the sample during heating. When the buffer inside the flask reached the appropriate temperature, 1 ml of spore suspension was added through the sampling port and a stopwatch started. At each sampling point, a 10-ml sample was removed and added to a sterile screwcap tube (20 by 150 mm) and immediately placed in ice water. Spores from three turkey isolates, 982-92, 1916-92, and 3280-94, were tested at 80 and 85°C. Samples were enumerated on brain heart infusion agar plates containing 0.1% sodium thioglycollate and 0.14% sodium bicarbonate *(20).* Plates were incubated anaerobically at 30°C for 72 h.

Inhibition studies. Studies of the turkey breast isolates were performed in TPGY broth to determine the effect of various additives and pH on growth rate and inhibition. Sodium lactate (Purac America Inc., Lincolnshire, Ill.), sodium diacetate (Purac), sodium nitrite (Sigma Chemical, St. Louis, Mo.), and sodium chloride (Fischer Scientific, Fair Lawn, N.J.) were used in these studies. Sodium lactate and sodium diacetate are used commercially to extend the shelf life of certain refrigerated processed meat and poultry products. The levels studied are listed as follows: (i) sodium chloride: 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0%; (ii) sodium diacetate: 0.05, 0.1, and 0.2%; (iii) sodium lactate: 0.5, 1.0, 1.5, and 2.0%; (iv) sodium nitrite: 50, 100, and 156 ppm; and (v) pH: 5.0, 5.5, 6.0, 6.5, and 7.0.

Cultures of each isolate were grown in TPGY for 48 h at 30°C, and 0.1-ml aliquots were inoculated into duplicate tubes of TPGY containing the concentrations of compounds listed above. Solutions of sodium lactate, sodium diacetate, and sodium nitrite were added as filter-sterilized solutions to freshly steamed tubes of TPGY to achieve the desired concentrations. Broth adjusted for NaCl content and pH was prepared and autoclaved. After inoculation, all tubes were sealed with Amojell to assure anaerobiosis. Absorbance was measured using a spectrophotometer (Perkin-Elmer model 35, Oak Brook, Ill.) with a wavelength setting of 600 nm. Measurements were taken initially and at regular intervals to measure growth.

Inoculated product studies. Two separate studies were conducted. Cooked turkey breast with the following ingredient listing: turkey breast, water, salt, modified food starch, dextrose, sodium phosphate, carrageenan, flavorings, and celery juice was obtained from a commercial establishment. This is the same type of product from which the isolates were initially recovered.

Three isolates were studied: 982-92, 1916-92, and 3280-94.

TABLE 1. *General characteristics of isolates from roast beef and cooked turkey breast*

Plant origin	Isolate[a]	Product	Storage time (days)	Hemolysis	Cell morphology	Spores
A	978-91	Roast beef	70	+	Very thick rods	Terminal
	979-91	Roast beef	100	+	Very thick rods	Terminal
	3-92	Roast beef	102	+	Very thick rods	Terminal
	12-92	Roast beef	60	+	Very thick rods	Terminal
B	1916-92	Turkey breast	60	−	Rods	Subterminal
	669-94	Turkey breast	68	−	Rods	Subterminal
	3280-94	Turkey breast	55	−	Rods	Subterminal
	6845-94	Turkey breast	53	−	Rods	Subterminal
	998-95	Turkey breast	90	−	Rods	Subterminal
C	982-92	Turkey breast	76	−	Rods	Subterminal

[a] Each isolate was obtained from a different production lot of product.

The first test studied growth rate at four temperatures: 10, 3.3, 0.5 and −3°C. Six kilograms of product was used for each isolate tested. The product was weighed, cut into small chunks, and mixed for 10 min in a Hobart mixer (Hobart Mfg. Co., Troy, Ohio) to achieve an emulsion-like consistency. A small amount of meat was removed and packaged to serve as an uninoculated control. Spore crops were removed from the freezer, heat shocked for 10 min at 75°C, and added to a target level of 10 spores/g. Spores were added slowly to the turkey dough and mixed for 15 min to ensure even distribution. This inoculated turkey was then packaged in barrier bags (Koch, Kansas City, Mo.), 100 g/bag, and vacuum sealed. The packaged product was then recooked to an internal temperature of 71.1°C for 5 min to destroy vegetative cell contaminants that may have been introduced during mixing and packaging. After cooking, the packages were removed, chilled in ice water, and placed at the desired temperatures. Two packages were used to determine initial levels of *C. ctm.* As the inoculum was low, counts were performed using a most probable number method with TPGY broth. Subsequent counts were performed every 10 days using most probable number and direct enumeration by plating on brain-heart infusion agar plates containing 0.1% sodium thioglycollate and 0.14% sodium bicarbonate. Plates were incubated anaerobically at 30°C for 72 h.

The second study examined the effects of two antimicrobials, sodium lactate *(10, 12)* and sodium diacetate *(10, 12, 21).* Eight kilograms of cooked turkey breast was used for each isolate to prepare four 2-kg variables. The first variable served as a control. Spores were added to a target level of 10/g and mixed for 15 min. The remaining three variables consisted of (i) 2.0% sodium lactate, (ii) 0.1% sodium diacetate, and (iii) 2.0% sodium lactate and 0.1% sodium diacetate. Each variable was mixed for 10 min with the additives and then inoculated and mixed as in the control. Each variable was subdivided into 100-g quantities, vacuum packaged, and treated as in the first study. The packages were placed at 3.3°C and sampled every 10 days for up to 90 days.

RESULTS

Isolation and characterization. All isolates were easily recovered from spoiled roast beef and turkey breast when using fluid thioglycollate broth at 10°C. The isolates fell into two groups based on the type of meat (beef or turkey), hemolysis on blood agar, and cell morphology. Ten isolates were chosen for characterization and study (Table 1). The last two digits of each isolate represent the year of isolation. Each isolate was from a separate spoilage incident.

All isolates are gram-positive, anaerobic, spore-forming rods. The roast beef isolates are short, fat rods that are β-hemolytic and capable of growing in the range of 3 to 15°C and not at 20°C or higher (Table 2). The turkey breast

TABLE 2. *Growth from 3.3 to 42°C during 28 days storage in TPGY broth*

Product	Isolate	Temperature (°C)							Optimum temperature (°C)[a]
		42	35	30	20	15	10	3.3	
Beef	978-91	−[b]	−	−	−	+	+	+	15
	979-91	−	−	−	−	+	+	+	15
	3-92	−	−	−	−	+	+	+	15
	12-92	−	−	−	−	+	+	+	15
Turkey	982-92	−	+	+	+	+	+	+	30
	1916-92	−	+	+	+	+	+	+	30
	669-94	−	+	+	+	+	+	+	30
	3280-94	−	+	+	+	+	+	+	30
	6845-94	−	+	+	+	+	+	+	30
	998-95	−	+	+	+	+	+	+	30

[a] Based on turbidity and gas production in TPGY broth.
[b] −, no growth; +, growth.

TABLE 3. *Biochemical characteristics of representative strains*

	12-92[a]	1916-92[b]
Characteristic		
Motility	+	+
Catalase	−	−
H_2S produced	+	+
Lecithinase produced	−	−
Lipase	+	−
Hemolysis	+	−
Gelatin liquefaction	−	−
Nitrate reduced	+	−
Starch hydrolysis	+	−
Acid from:		
Fructose	+	+
Galactose	+	−
Glucose	+	+
Lactose	−	−
Maltose	−	−
Raffinose	+	−
Rhamnose	−	−
Sucrose	+	−

[a] Roast beef isolate.
[b] Turkey breast isolate.

isolates are rods, negative for β-hemolysin, and with a much wider temperature range for growth (i.e., 3 to 35°C). Growth at temperatures below 3°C was not evaluated in broth. The roast beef isolates have an optimum growth temperature of about 15°C, while the isolates from turkey have an optimum growth temperature of about 30°C.

The results of the carbohydrate fermentations and biochemical tests are listed in Table 3.

Identification. Six isolates from spoiled turkey breast were identified by Anatec, Inc. (Blacksburg, Va.) as *C. ctm,* an interim designation assigned to a group of clostridia previously isolated from cooked turkey meat and submitted to the Virginia Polytechnic Institute Anaerobe Laboratory from another source.

Toxicity. The four isolates tested from turkey, 982-92, 1916-92, 3280-94, and 998-95, were not lethal to mice.

Heat resistance of spores. Heat resistance (D values) determined on spore crops from turkey isolates 982-92, 1916-92, and 3280-94 at 80 and 85°C is reported in Table 4. These values are comparable to results obtained by Segner *(20)* in which a psychrotrophic *Clostridium* sp. isolated from crabmeat had D values of 30 and 15.6 min at 82.2 and 85°C, respectively.

TABLE 4. *D values of spores of turkey isolates in phosphate buffer*

Heating temperature	D values (min) for isolate:		
	982-92	1916-92	3280-94
80°C	37[a]	17	28.5
85°C	17.5	8	9

[a] Average of two trials.

TABLE 5. *Absorbance[a] of* C. ctm *isolates at varying pH in TPGY broth incubated at 30°C*

Time (h)	Control (6.8)	pH				
		5.0	5.5	6.0	6.5	7.0
0	0	0	0	0	0	0
4	0	0	0	0	0	0
21	0.10	0	0	0.03	0.08	0.07
24	0.12	0	0	0.02	0.08	0.14
28	0.24	0	0	0.05	0.18	0.24
44	0.33	0	0	0.24	0.28	0.33
48	0.31	0	0	0.23	0.28	0.32
72	0.32	0	0.16	0.24	0.30	0.32

[a] Wavelength setting at 600 nm; mean of two replicates.

Inhibition studies. Isolates 982-92, 1916-92, and 3280-94 reached stationary phase by 48 h at 30°C in TPGY with no additives. Because the data generated for the three isolates yielded the same or similar results, Tables 5 through 9 represent typical results that were obtained.

Growth in TPGY broth adjusted to pH values of 5.0 to 7.0 indicates that the most rapid growth occurred at about pH 6.5 to 7.0 (Table 5). As the pH was decreased to pH 6.0 and below, rate of growth also decreased. All three isolates were inhibited at pH 5.0.

Growth was inhibited by 2.5% NaCl and higher during incubation at 30°C for 72 h (Table 6). Each 0.5% incremental increase of NaCl from 1.0 to 2.5% NaCl resulted in greater inhibition.

Sodium nitrite was a very effective inhibitor (Table 7). Growth was prevented at the 100- and 156-ppm levels for all three isolates during the 72-h incubation, thereby explaining why spoilage of cured turkey breast products has not been observed.

Growth rates for varying levels of sodium lactate appear in Table 8. None of the levels tested prevented growth; however, each 0.5% increase resulted in slower growth. All three isolates responded similarly at each level tested.

Table 9 shows the effect of sodium diacetate. Growth was slightly slower for all three isolates at the 0.2% level.

TABLE 6. *Absorbance[a] of* C. ctm *isolates at varying sodium chloride levels in TPGY broth incubated at 30°C*

Time (h)	Control	Sodium chloride level (%)						
		1	1.5	2	2.5	3	3.5	4
0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
21	0.10	0.04	0.04	0.03	0.01	0	0	0
24	0.12	0.03	0.03	0.01	0	0	0	0
28	0.24	0.05	0.02	0	0	0	0	0
44	0.33	0.29	0.15	0.04	0	0	0	0
48	0.31	0.29	0.22	0.03	0	0	0	0
72	0.32	0.29	0.25	0.24	0.01	0	0	0

[a] Wavelength setting at 60 nm; mean of two replicates.

TABLE 7. *Absorbance[a] of* C. ctm *isolates at varying sodium nitrite levels in TPGY broth incubated at 30°C*

		Nitrite level (ppm)		
Time (h)	Control	50	100	156
0	0	0	0	0
4	0	0	0	0
21	0.10	0	0	0
24	0.12	0	0	0
28	0.24	0	0	0
44	0.33	0.14	0	0
48	0.31	0.13	0	0
72	0.32	0.14	0	0

[a] Wavelength setting at 600 nm; mean of two replicates.

TABLE 8. *Absorbance[a] of* C. ctm *isolates at varying sodium lactate levels in TPGY broth incubated at 30°C*

		Sodium lactate level (%)			
Time (h)	Control	0.5	1	1.5	2
0	0	0	0	0	0
4	0	0	0	0	0
21	0.10	0.06	0.03	0.03	0.02
24	0.12	0.05	0.04	0.02	0.01
28	0.24	0.10	0.03	0.01	0.01
44	0.33	0.28	0.25	0.18	0.07
48	0.31	0.27	0.25	0.22	0.11
72	0.32	0.26	0.25	0.26	0.22

[a] Wavelength setting at 600 nm; mean of two replicates.

Isolates 3280-94 and 1916-92 did not show a difference between the control and the 0.05% and 0.1% variables.

Inoculated product study I. Table 10 shows the growth of three isolates in cooked turkey breast stored at different temperatures. Growth was most rapid at 10°C and slower as the temperature decreased.

For isolate 982-92 at 10°C, exponential growth was apparent between 40 and 50 days. An H_2S odor was detected upon opening the packages at 50 days. At 60 days the product was spoiled with pink discoloration and a strong H_2S odor. At 3.3°C, pink discoloration and H_2S odor was apparent at 70 days. Spoilage did not occur at 0.5 and −3°C in 80 days; however, growth did occur.

For isolates 1916-92 and 3280-94 at 10°C, exponential phase occurred between 10 and 20 days. By 40 days an H_2S odor and pink discoloration were detected. At 3.3°C exponential phase occurred between 40 and 60 days. Pink discoloration and a slight H_2S odor were detected by 70 days. No growth or spoilage occurred at 0.5 or −3°C. Uninoculated control packages sampled at 30, 60, and 90 days yielded no detectable microflora and displayed no signs of spoilage.

Inoculated product study II. The same three isolates were used to evaluate the effect of sodium lactate and/or sodium diacetate in cooked turkey stored at 3.3°C (Table 11). The results varied with the isolate and the additive, indicating a need for further research.

Growth of isolate 982-92 was apparent at 40 days for the untreated control samples. Signs of spoilage, including pink discoloration and H_2S odor were evident by day 50. All three treatments tested prevented growth of this isolate throughout 80 days of storage.

Isolate 1916-92 performed quite differently from the other isolates by being able to multiply in product containing 2.0% sodium lactate. In fact, growth occurred more rapidly with the addition of sodium lactate. At 40 days, samples treated with sodium lactate were considered spoiled. Control product became pink by 70 days, with noticeable H_2S odor at 80 days. Addition of 0.1% sodium diacetate yielded variable results compared to the control. The sodium lactate/sodium diacetate combination was comparable to the control.

Growth of isolate 3280-94 was apparent at 40 days in the control product, with pink discoloration and H_2S odor at 50 days. Although growth did occur in all treatments, it was slower than in the control. Growth was delayed in product containing 0.1% sodium diacetate, but pink discoloration was noticeable at 80 days. The sodium lactate and sodium lactate/sodium diacetate variables more effectively delayed growth and prevented spoilage through 80 days.

DISCUSSION

Broda et al. *(4, 5)* have proposed that psychrotrophic clostridia associated with meat spoilage be divided into two groups based on their optimum growth temperature: group 1, 15 to 20°C and group 2, 25 to 30°C. It is clear from the characterization of the isolates recovered from cook-in-bag roast beef and turkey breast, that two distinct species of psychrotrophic clostridia were responsible for the spoilage of these products. *C. laramie* (group 1), the isolates from roast beef, is a recently recognized species that is identifiable through published information *(16).* The isolates from turkey breast have been temporarily designated *C. ctm* and would meet the criteria for group 2. This research concentrated on the latter isolates and their control.

The isolates are nontoxigenic, psychrotrophic, anaerobic sporeformers with similar growth characteristics in broth media. The results provide some helpful information to prevent or reduce the growth of these organisms. Sodium

TABLE 9. *Absorbance[a] of* C. ctm *isolates at varying sodium diacetate levels in TPGY broth incubated at 30°C*

		Sodium diacetate level (%)		
Time (h)	Control	0.05	0.1	0.2
0	0	0	0	0
4	0	0	0	0
21	0.10	0.06	0.04	0.04
24	0.12	0.06	0.04	0.03
28	0.24	0.10	0.09	0.03
44	0.33	0.32	0.31	0.20
48	0.31	0.31	0.30	0.23
72	0.32	0.32	0.32	0.28

[a] Wavelength setting at 600 nm; mean of two replicates.

TABLE 10. *Growth of* C. ctm *isolates at varying temperatures in cooked turkey*

		C. ctm (log_{10}CFU/g) at storage time (days)								
Isolate	Temperature	0	10	20	30	40	50	60	70	80
982-92	−3	1.3[a]	2.1	2.2	1.8	1.8	2.0	2.3	2.6	2.9
	0.5	1.3	2.7	2.3	2.8	2.1	2.5	2.8	3.3	4.1
	3.3	1.3	2.4	2.7	2.9	3.1	3.5	3.8	4.2	4.6
	10.0	1.3	2.1	1.3	2.4	3.1	4.3	5.1	6.2	7.7
1916-92	−3	0.95	1.9	1.0	0.7	0.6	0.9	1.4	1.4	1.4
	0.5	0.95	1.8	0.5	0.9	0.7	1.3	1.8	1.4	0.6
	3.3	0.95	1.3	0.6	1.3	3.6	4.0	4.4	5.1	5.7
	10.0	0.95	1.5	4.8	6.7	7.6	7.8	8.1	8.4	8.2
3280-94	−3	1.3	1.2	0.5	0.8	1.1	1.2	1.5	1.4	1.2
	0.5	1.3	1.2	0.5	0.6	1.1	1.2	1.5	1.4	1.0
	3.3	1.3	1.6	0.95	2.8	0.9	2.5	4.3	5.0	5.7
	10.0	1.3	1.7	5.1	6.6	6.9	7.3	7.7	8.4	8.2

[a] Mean of two replicates.

nitrite at 100 and 156 ppm prevented growth of all three isolates while 50-ppm nitrite was also quite inhibitory against two of the three isolates. These results explain why cured cook-in-bag turkey products have never displayed spoilage typical of *C. ctm.* Sodium lactate and sodium diacetate at levels of 2.0% and 0.2%, respectively, effectively reduced growth of these organisms in broth. Growth in 3.0% NaCl prevented growth of these isolates. The products that these isolates were recovered from, however, are low-salt products containing NaCl levels between 1.5 and 2.0% NaCl. Thus, to maintain a natural tasting, low-salt uncured product, other antimicrobials such as sodium lactate or sodium diacetate may be considered.

The first of two inoculated product studies investigated the effect of low temperature storage. At 10 and 3.3°C all three isolates multiplied and spoiled the product. At −3 and 0.5°C isolate 982-92 increased approximately 1 and 2 logs, respectively, whereas the other two isolates did not multiply during 90 days storage. These data indicate that temperatures of less than 3°C during storage and distribution would reduce the risk of spoilage.

The second inoculated product study involved adding sodium lactate and sodium diacetate alone and in combination. Differences were observed in the growth of the isolates. Isolate 982-92 was inhibited by all three treatments. Growth of isolate 3280-94 was delayed by all three treatments, with 2.0% sodium lactate and the combination of 2.0% sodium lactate and 0.1% sodium diacetate demonstrating greatest inhibition. Isolate 1916-92 grew rapidly in product containing 2.0% sodium lactate, compared with the control product. The two other treatments resulted in delayed growth, with 0.1% sodium diacetate offering a slightly greater inhibitory effect. The data indicate that additional research is needed; however, use of 0.1% sodium diacetate should offer some protection against spoilage.

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TABLE 11. *Growth of* C. ctm *isolates in cooked turkey containing sodium lactate and diacetate and stored at 3.3°C*

		C. ctm (log_{10}CFU/g) at storage time (days)								
Isolate	Variable[a]	0	10	20	30	40	50	60	70	80
982-92	A	1.1[b]	1.8	2.1	3.1	5.5	3.7	3.6	4.4	7.7
	B	1.1	0.95	1.7	2.1	2.0	1.8	2.1	1.9	2.2
	C	1.1	1.6	1.7	2.0	1.8	1.9	2.3	2.0	1.8
	D	1.1	1.0	2.2	1.9	2.0	2.3	1.6	1.8	2.8
1916-92	A	1.9	2.4	1.9	3.1	5.1	3.3	3.5	3.6	4.0
	B	1.9	1.8	2.0	2.8	2.7	2.4	5.3	5.8	5.2
	C	1.9	3.0	3.9	5.4	6.6	7.3	7.6	7.4	8.0
	D	1.9	2.4	2.5	3.3	3.8	3.8	5.8	4.2	5.1
3280-94	A	0.95	2.5	3.0	4.4	6.5	5.9	6.2	7.5	7.5
	B	0.95	3.0	3.0	3.0	2.6	5.3	5.1	4.7	5.0
	C	0.95	2.1	1.6	3.2	3.8	2.0	2.9	1.8	3.2
	D	0.95	1.8	2.9	2.1	2.3	2.3	1.9	2.0	3.7

[a] A, control; B, 0.1% sodium diacetate; C, 2.0% sodium lactate; D, 0.1% sodium diacetate + 2.0% sodium lactate.
[b] Mean of two replicates.

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