

# Lactobacilli and Tartrazine as Causative Agents of Red-Color Spoilage in Cucumber Pickle Products

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**ABSTRACT:** The cucumber pickling industry has sporadically experienced spoilage outbreaks in pickled cucumber products characterized by development of red color on the surface of the fruits. *Lactobacillus casei* and *Lactobacillus paracasei* were isolated from 2 outbreaks of this spoilage that occurred about 15 y apart during the last 3 decades. Both organisms were shown to produce this spoilage when inoculated into pickled cucumbers while concomitantly degrading the azo dye tartrazine (FD&C yellow nr 5). This food dye is used as a yellow coloring in the brine cover solutions of commercial pickled cucumber products. The red color does not occur in the absence of tartrazine, nor when turmeric is used as a yellow coloring in the pickles. Addition of sodium benzoate to the brine cover solutions of a pickled cucumber product, more specifically hamburger dill pickles, prevented growth of these lactic acid bacteria and the development of the red spoilage.

**Keywords:** azo dye, benzoic acid, FD&C yellow nr 5, *Lactobacillus casei*, *Lactobacillus paracasei*

## Introduction

The pickled vegetable industry in the United States has occasionally reported spoilage in which a red coloration develops in fermented products such as hamburger dill cucumber pickles. There have been approximately 6 cases of red-colored cucumbers reported to us in the last 30 y. Specifically, it has been observed that a red pigment is formed in the fermented cucumber skins about 1 wk postpacking (Figure 1). This kind of spoilage primarily affects the products packed in bulk, and although it is a recurrent problem it has caused minimal economic losses for the industry.

Approximately 30 y ago scientists in this laboratory initiated studies to determine the cause(s) of the spoilage on packed cucumbers. They isolated and identified *Lactobacillus casei* as a causative agent of the red coloration and observed that FD&C yellow nr 5 or tartrazine, which is commonly added as a yellow coloring in commercial cucumber pickles, had a role in the development of the red color. About 15 y later, another organism was isolated from an outbreak of this spoilage and maintained in our culture collection. More recently another outbreak of this red-color spoilage was reported, renewing research interest. The objective of this report is to describe our investigation of the most recent outbreak and relate the results to the earlier outbreaks. The observations presented here confirm the early findings identifying *L. casei* and tartrazine as the causative agents of the red-color spoilage, and describe methods to prevent its formation.

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## Materials and Methods

### Isolation of the causative agent

Two samples of red-colored hamburger dill cucumber pickles were obtained from the company that observed the spoilage. These were designated as sample I and II. The isolation method was to remove a segment of the skin of the red-colored cucumber pickles with a sterile scalpel. The samples of the skin tissue (1.0 g) were mixed with 9-mL saline solution and vortexed for 30 s. The vortexed samples were stomached (Seward Stomacher 400, Ohio, U.S.A.) in 6" × 4.5" filter stomacher bags for 1 min at medium speed. Aliquots of 100 μL from the filtered stomacher bags were spread plated on plate count agar (PCA), yeast malt extract agar (YMA), and *Lactobacilli* deMan, Rogosa, and Sharpe agar (MRSA) (Becton, Dickinson and Co., N.J., U.S.A.) and incubated at 30 °C for 24 h. Pure cultures of the colonies presenting the predominant colony morphologies were obtained by restreaking in MRSA. Frozen stocks of the isolates were prepared in MRS broth (Becton, Dickinson and Co.) containing 10% glycerol (Fisher Scientific, Pittsburg, Pa., U.S.A.).

### Reproduction of the spoilage

Nonspoiled hamburger dill pickles and cover brine solutions containing tartrazine coloring were obtained from a commercial source. Fermented hamburger dill pickles were transferred from the original jars to sterile 8 oz. jars using aseptic techniques in a microbiological hood (Nuair Biological Safety Cabinet, Plymouth, Minn., U.S.A.). The tartrazine brine was added and jars were closed with commercial lug caps in which a rubber septum was inserted to allow for inoculation and sampling of the jars with sterile syringes. The lids were heated in boiling water for 10 to 15 s to soften the sealing compound and immediately applied to the filled jars. Jars were incubated at 30 °C for 2 d to allow equilibration of components between the cucumbers and cover brine solution. Samples of the equilibrated brines were collected using 1-mL tuberculin syringes (Fisher Scientific) and plated on MRSA, PCA, and YMA. Jars were inoculated with the microorganisms isolated from

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red-colored cucumbers. Preparation of cultures to inoculate the jars of pickles was done by streaking bacteria from frozen stock culture on MRSA, and incubating the plates for 48 h at 30 °C. Single colonies were transferred to 10-mL MRS broth, and incubated at 30 °C for 36 h. Cultures were centrifuged, washed, and resuspended twice with saline solution prior to the inoculation of the jars. Isolates were individually inoculated through the lid septum of the cucumber jars to achieve 10<sup>3</sup> CFU/mL in each jar. Control jars were not inoculated. All jars were incubated at 30 °C for 3 wk. Formation of the red coloration was visually monitored. Jars in which the red-color spoilage developed were used for the reisolation of the causative agent(s) as described previously.

### Identification of isolates that caused red-colored spoilage

The bacterial isolates LA1133 and LA0471 and the bacterial reisolate LA1133-R were classified using partial 16S rRNA gene sequencing and also identified by biochemical tests using the API 50 CHL system (Biomérieux Inc., N.C., U.S.A.). The partial 16S rRNA gene sequence was obtained from a fragment amplified by PCR using chromosomal DNA as a template. Chromosomal DNA was obtained using a genomic DNA purification kit (Promega Corp., Madison, Wis., U.S.A.). The PCR mixture contained Platinum PCR SuperMix (Invitrogen Corp., Carlsbad, Calif., U.S.A.), chromosomal DNA, and the Rd1 (5'-GTC TCA GTC CCA ATG TGG CC-3'), and Ru2 (5'-AGA GTT TGA TCC TGG CTC AG-3') primers (Barrangou and others 2002) (Sigma-Genosys, Saint Louis, Mo., U.S.A.) or the UF1 (5'-AGAGTTTGATCTGGCTCAG-3') and UR1 (5'-GCTGGCAGCTAGTTAGCC-3') (Steele 2007) (Integrated DNA Technologies, Coralville, Iowa, U.S.A.) primers. The PCR cycle consisted of 4 min at 95 °C followed by 30 cycles of 30 s at 95 °C, 30 s at 61 °C, and 30 s at 72 °C, with a final extension step of 7 min at 72 °C and stored at 4 °C until used. The PCR products were purified (Qiagen PCR purification kit, Valencia, Calif., U.S.A.) and sequenced by Davis Sequencing (Davis, Calif., U.S.A.) using an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, Calif., U.S.A.). The sequences obtained were subjected to the basic local alignment search tool (BLAST) (Altschul and others 1990) in the GenBank database (Benson and others 1997) to determine the identities of the strains.

The API 50 CHL system was used following the manufacturers instructions.

### Role of tartrazine on the development of the red-colored spoilage

The role of tartrazine on the development of the red-colored spoilage was determined by observing the effect of the addition of tartrazine, turmeric, or no coloring on the formation of red color on the surface of cucumbers. Turmeric is a natural spice used to color cucumber pickles that contains the yellow pigment curcumin. Brines containing tartrazine, turmeric, or no coloring agent were obtained from a commercial source. Size 2B cucumbers (44- to 51-

mm diameter) were obtained locally and packed in 24 oz. jars using a 50:50 w/v ratio. Jars were closed with lids with a rubber septum inserted as described above. Jars were incubated at ambient temperature (23 ± 3 °C) for 2 d prior to inoculation to allow for equilibration of components between the cucumbers and cover brine solution.

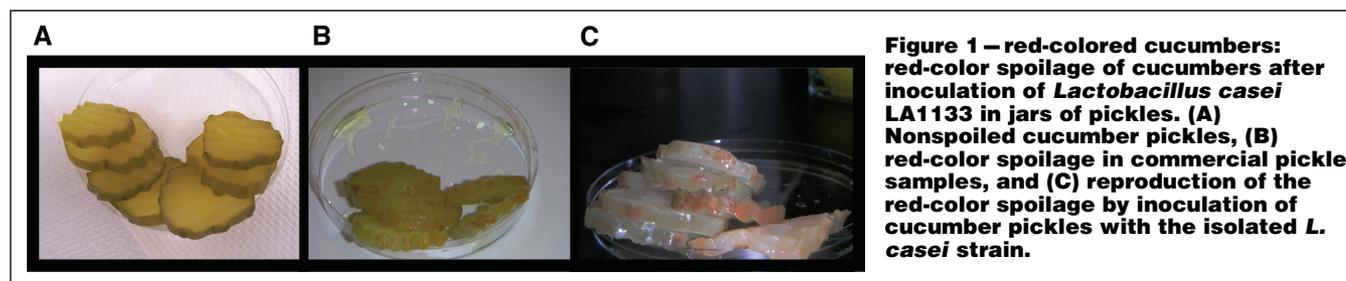
Jars were inoculated to 10<sup>4</sup> CFU/mL of either *L. casei* LA1133 or *L. paracasei* LA0471. Inocula were prepared as described previously. Inoculated jars were incubated at 30 °C for 2 wk, and monitored daily for the appearance of turbidity, the development of the red color on the cucumber skins, and the disappearance of yellow color from the brines. Additionally, disappearance of tartrazine was monitored spectrophotometrically at 427 nm from brine supernatants twice a week using a Varian Cary 300 Bio UV-Visible Spectrophotometer (Palo Alto, Calif., U.S.A.).

### Utilization of tartrazine by lactobacilli

Size 2B cucumbers were packed as described previously. Brine containing 0.24 mM tartrazine (Sigma-Aldrich Co.) was prepared as described below. Jars were pasteurized by heating in a water bath in a steam jacketed kettle to a temperature of 74 °C at the slowest heating point in the jars. The temperature was maintained for 15 min. Subsequently, jars were cooled to 40 °C in cold tap water before removing them from the steam-jacketed kettle. Jars were incubated at 23 °C for 2 d prior to inoculation to allow for equilibration of components between the cucumbers and the cover brine solution. Jars were inoculated to 10<sup>4</sup> CFU/mL with either *L. casei* LA1133 or *L. paracasei* LA0471 as described previously. Noninoculated jars were utilized as negative controls. All jars were incubated at 30 °C for the extent of the experiment. The time course for growth of *L. casei* and *L. paracasei* was monitored by measuring OD<sub>600</sub> with a Varian Cary 300 Bio UV-Visible Spectrophotometer in brine samples taken after shaking the jars. Disappearance of tartrazine was measured by the loss in absorbance at 427 nm. Absorbance was measured from culture supernatants.

### Effect of pH on the development of red spoilage

The pH of the cucumber jars was adjusted to 3.9, 3.5, 2.9, 2.3, or 1.8 ± 0.1 to observe the effect of this variable on the development of the red-colored spoilage. Amounts of NaOH or HCl to be added to the jars for the adjustment of the equilibrated pH were determined by titration of cucumber slurry. Cucumber slurry was prepared using a Waring blender (Torrington, Conn., U.S.A.), and a 50:50 (w/v) ratio of size 2B cucumbers and cover brine solution. The cover brine contained 2.28 M sodium chloride (Morton canning and pickling salt), 40 mM calcium chloride, 106 mM acetic acid, and 0.24 mM tartrazine (Sigma-Aldrich Co.). Size 2B cucumbers were packed in 24 oz. jars as described previously, with the addition of the NaOH or HCl to achieve the desired pH. Jars were closed with lids with a rubber septum inserted as described above. Brine from each jar was sampled and the equilibrated brine pH was measured using an IQ240 pH meter (IQ Scientific Instruments,



**Figure 1 – red-colored cucumbers: red-color spoilage of cucumbers after inoculation of *Lactobacillus casei* LA1133 in jars of pickles. (A) Nonspoilaged cucumber pickles, (B) red-color spoilage in commercial pickle samples, and (C) reproduction of the red-color spoilage by inoculation of cucumber pickles with the isolated *L. casei* strain.**

San Diego, Calif., U.S.A.) prior to inoculation. Jars were inoculated with either *L. casei* LA1133 or *L. paracasei* LA0471 through the lids septum to  $10^4$  CFU/mL. Inocula were prepared as described previously. Inoculated jars were incubated at 30 °C and monitored daily for turbidity and the development of the red color on the cucumber surfaces. The disappearance of the tartrazine was measured spectrophotometrically at 427 nm from brine supernatants using a Varian Cary 300 Bio UV-Visible Spectrophotometer.

### Spectrophotometric detection of a tartrazine utilization product

MRS broth cultures of the strains isolated (24 h, 30 °C) were transferred to modified chemically defined media (mCDM) containing 0 or 0.24 mM tartrazine (Sigma-Aldrich Co.). The mCDM was prepared as described by Christensen and Steele (2003) with the following modifications: (1) all individual amino acids were replaced by 10.0 g of Bacto Casitone (Becton Dickinson and Co., Sparks, Md., U.S.A.) per liter and (2) 2.5 mg pyridoxamine dihydrochloride (Sigma-Aldrich Co.) was added per liter. Substitution of individual amino acids by Bacto Casitone was essential for growth of the lactobacilli. The mCDM carbohydrate content was adjusted to 10 mM glucose. Cultures were incubated at 30 °C for 4 d. Disappearance of tartrazine and the appearance of a tartrazine utilization product were measured for the 4 d of incubation by spectrophotometry at 427 and 560 nm, respectively, from culture supernatants using a Varian Cary 300 Bio UV-Visible Spectrophotometer.

### Prevention of the development of red-color spoilage in commercial hamburger dill pickles

Jars from a single production lot of hamburger dill pickles were obtained from 2 processors. Product A did not contain preservatives. Product B contained sodium benzoate as preservative. Major components of these products were analyzed. Concentrations of acetic acid, lactic acid, benzoic acid, and sorbic acid were determined using a 10-cm Bio-Rad Fast Acid column (Bio-Rad Laboratories, Hercules, Calif., U.S.A.) and a Thermo Separations HPLC system (Waltham, Mass., U.S.A.). The column was heated to 75 °C and eluted isocratically with 0.03 N sulfuric acid at a flow rate of 1.0 mL/min. Crystalline L-lactic acid, sodium benzoate, potassium sorbate (Sigma-Aldrich, Co.) and reagent grade glacial acetic acid (Fisher Scientific) were dissolved in water for standards. Samples were prepared by centrifuging the brines at 14000 g, 30 °C for 5 min in a 1.5-mL microcentrifuge tube to remove particles and then diluting them 25-fold into autosampler vials. The calcium concentration was determined by the spectrophotometric method of Gindler and King (1972) using a 100-fold diluted brine samples. Absorbance readings at 612 nm were made using a Varian Cary 300 Bio UV-Visible Spectrophotometer. Results were expressed as percent of calcium chloride. Chloride concentration was measured by the Fajan method in which brine samples were titrated with 0.171 N silver nitrate solution using dichlorofluorescein as an end-point indicator (Skoog and others 1996). The chloride concentra-

tion was expressed as percent sodium chloride. Tartrazine was determined by measuring the absorbance of cultures or brines supernatants at 427 nm using a Varian Cary 300 Bio UV-Visible Spectrophotometer.

The hamburger dill pickle jars were opened aseptically and the lids replaced with lids containing a rubber septum, which were heated in boiling water before reclosing the jars. Some of the jars were diluted 20% with water or supplemented with sodium benzoate, potassium sorbate, salt, calcium, or acetic acid as indicated on Table 1. The concentrations of the measured major components on the original pickle products and of the added components are shown in Table 1. Following supplementation, the jars were held at room temperature ( $23 \pm 3$  °C) for 48 h with occasional mixing to allow equilibration of components between cucumbers and the cover brine solutions before inoculation. After equilibration jars of each treatment were inoculated with a cocktail of  $5 \times 10^5$  CFU/mL *L. casei* LA1133 and  $5 \times 10^5$  CFU/mL *L. paracasei* LA0471 prepared as described previously and incubated at 30 °C for 2 wk. In addition triplicate noninoculated jars of each treatment were incubated as negative controls. Viable bacteria were determined after 2 wk plating on MRSA using an Autoplate 4000, and Qcount (Spiral Biotech, Norwood, Mass., U.S.A.). Formation of the red spoilage was visually monitored daily for 2 wk.

## Results

### Identification of bacteria that cause red-colored spoilage

Four outbreak isolates were obtained from MRS plates. The 4 isolates, including a Gram-positive bacterium (LA1133), a Gram-negative bacterium, and 2 yeasts, were tested for their ability to produce red-colored spoilage in jars of pasteurized hamburger dill cucumber pickles. Although all 4 isolates grew in the inoculated jars, only the isolate designated as LA1133 was able to produce the red-color spoilage (Figure 1). No growth was observed in the noninoculated jars. A microorganism with the same colony morphology shown by LA1133 was reisolated from the jars utilized to reproduce the spoilage in the laboratory. The organism that was reisolated from the red-color spoiled jar was designated as LA1133-R. The Gram-positive rods isolated and identified as the causative agents of red-colored spoilage, LA1133 and LA1133-R, were both identified as *Lactobacillus casei* ATCC393 on the basis of fermentation patterns with the API 50CHL system and by sequence homology (99% to 100%) of 300 or 500 bp fragments of the 16S rRNA gene. On the same basis strain LA0471, which was isolated from pickles that exhibited red spoilage in the 1990s, was identified as *Lactobacillus paracasei*. This strain was also capable of reproducing the red-color spoilage in the presence of tartrazine.

### Role of tartrazine in the red-colored spoilage

Red color was only formed in pickles that were inoculated with *L. casei* LA1133 and *L. paracasei* LA0471 and that contained tartrazine.

**Table 1 – Composition of hamburger dill pickles inoculated with a cocktail of *Lactobacillus casei* LA1133 and *Lactobacillus paracasei* LA0471**

Treatment	NaCl (%)	CaCl <sub>2</sub> (%)	Acetic acid (mM)	Lactic acid (mM)	Sorbic acid (%)	Benzoic acid (%)	Tartrazine (mM)	pH
A:1	2.8	0.5	287	ND <sup>a</sup>	ND	ND	0.25	3.6
A:2	2.8	0.5	287	ND	0.1	ND	0.25	3.6
A:3	2.8	0.5	287	ND	ND	0.10	0.25	3.6
A:4	2.8	0.5	287	ND	0.05	0.05	0.25	3.6
A:5	3.5	0.8	300	ND	0.1	ND	0.25	3.5
B:6	2.0	0.6	187	50.81	ND	0.07	0.25	3.3
B:7	1.6	0.5	150	40.65	ND	0.06	0.20	3.3

<sup>a</sup>ND = not detected

Pickles to which no coloring or turmeric was added as a yellow coloring did not develop the red-colored spoilage when inoculated with the causative bacterial cultures.

### Utilization of tartrazine by lactobacilli

Utilization of tartrazine by *L. casei* LA1133 and *L. paracasei* LA0471 was initiated during the exponential phase of growth in inoculated jars of pasteurized, acidified cucumbers (Figure 2). *L. casei* LA1133 grew at a rate of 0.052/h, which was more than double the rate of *L. paracasei* LA0471 (0.023/h) in the acidified cucumbers. However, *L. paracasei* degraded 80% tartrazine in less than 13 d, while *L. casei* only degraded 35% of the initial tartrazine in 17 d (Figure 2). Tartrazine was degraded to a limited extent (20%) in the noninoculated jars.

### pH effect on the production of red-color spoilage

Cucumbers packed at equilibrated pH of 3.9 and  $3.5 \pm 0.1$  supported bacterial growth, disappearance of tartrazine (Figure 3), formation of the red-colored spoilage, and a drop in the initial equilibrated pH (data not shown). *L. casei* LA1133 and *L. paracasei* LA0471 utilized tartrazine to the same extent after 25 d of incubation at these pH values. Although cucumbers packed at equi-

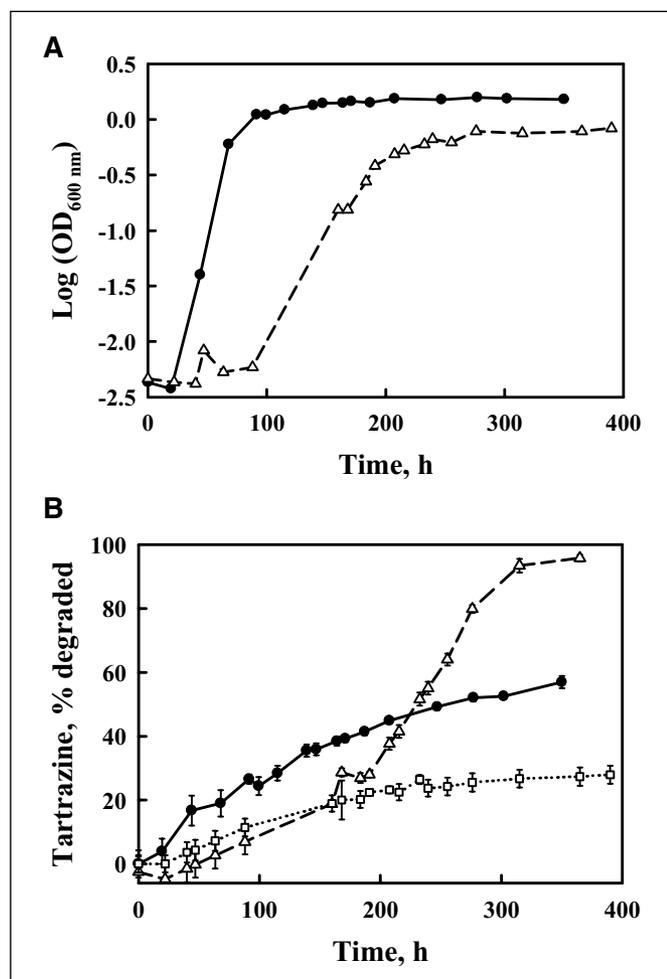
librated pH of 2.9, 2.3, and 1.8 supported some bacterial growth, tartrazine did not disappear (Figure 3), the pH did not decline as a result of bacterial growth, nor did the red-colored spoilage develop.

### Detection of a tartrazine utilization product

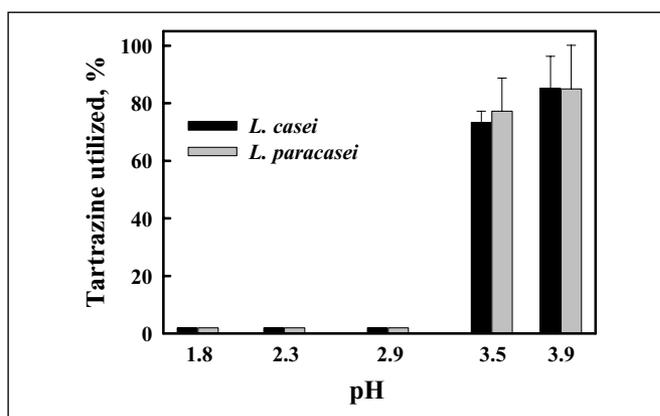
Utilization of tartrazine in mCDM medium in the absence of cucumbers was also investigated. Tartrazine was utilized to a limited extent by both spoilage organisms in this growth medium. Along with the decline in absorbance at 427 nm (Figure 4A), there was a small increase in absorbance with a maximum intensity at 560 nm (Figure 4B). A decline in absorbance at 427 nm was not observed in mCDM acidified with lactic acid or hydrochloric acid. The spectrum of the culture medium after tartrazine utilization from 480 to 700 nm is shown in Figure 5. Since this increase in absorption at 560 nm did not occur if tartrazine was not added to the medium, it is presumed to be a product of tartrazine utilization. The increase in absorbance at 560 nm is much less than the loss in absorbance at 427 nm due to tartrazine utilization. It is not known if the absorbance intensity of the product is much less than that of tartrazine or if the compound with an absorption maximum at 560 nm is a minor product of tartrazine utilization.

### Development and prevention of red-color spoilage in commercial hamburger dill pickles

Table 2 shows the results obtained from the 7 treatments described in Table 1. It shows that jars subjected to treatment A:1, which were inoculated with a mixture of *L. casei* LA1133 and *L. paracasei* LA0471, developed the red-color spoilage. Treatment A:1 consisted of hamburger dill pickles that contained no sodium benzoate or potassium sorbate (product A). Spoilage also was observed if potassium sorbate equivalent to 0.1% sorbic acid was added to the commercial product, as in treatment A:2. However, the extent of tartrazine utilization by the lactobacilli was reduced from 77.9% to 20.5% and the red-color spoilage developed on only about 1.5% of the pickle slices in a jar. Bacterial growth was not inhibited by sorbic acid. Addition of sodium benzoate equivalent to 0.1% benzoic acid, as in treatment A:3, prevented bacterial growth, disappearance of tartrazine, and development of the red spoilage.



**Figure 2—Utilization of tartrazine coloring by lactobacilli in cucumber pickles: (A) growth of *Lactobacillus casei* LA1133 (•) and *Lactobacillus paracasei* LA0471 (Δ) in cucumber jars, and (B) tartrazine utilization in jars inoculated with *L. casei* LA1133 (•) and *L. paracasei* LA0471 (Δ), and noninoculated jars (□). Disappearance of tartrazine was followed spectrophotometrically at 427 nm.**



**Figure 3—Effect of equilibrated pH on the development of red-colored cucumber skins: acidified cucumbers were inoculated with *Lactobacillus casei* LA1133 (black bars) and *Lactobacillus paracasei* LA0471 (gray bars). The pH of the cover solutions was adjusted to obtain an equilibrated pH of either 3.9, 3.5, 2.9, 2.3, or  $1.8 \pm 0.1$ . Percentage of tartrazine utilized was determined from the difference of the initial and final absorbance at 427 nm. Absorbance measurements were made after 25 d of incubation.**

Treatment A:4, which contained a mixture of 0.05% sodium benzoate calculated as benzoic acid and 0.05% potassium sorbate calculated as sorbic acid, prevented formation of the red-color spoilage. Counts of viable bacteria were 5-logs higher than when 0.1% benzoic acid was added. Although cells survived for a longer period of time in these jars, no bacterial growth was observed. Treatment A:5, which consisted of increased concentrations of

sodium chloride, calcium chloride, and acetic acid, along with 0.1% sorbic acid, did prevent both bacterial growth and red spoilage.

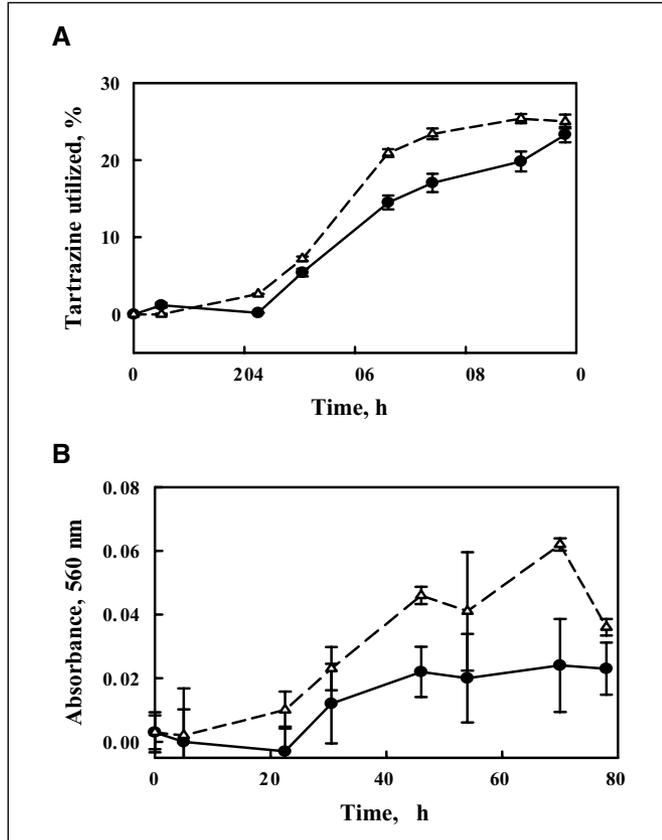
Red spoilage did not occur in commercial product B, which contained 0.071% benzoic acid (treatment B:6), even though it had lower concentrations of NaCl and acetic acid than product A (treatment A:1). There was, however, about a 10% loss in tartrazine. Visible spoilage still did not occur when product B was diluted by 20% with water to reduce the concentration of all components (treatment B:7), including benzoic acid.

### Discussion

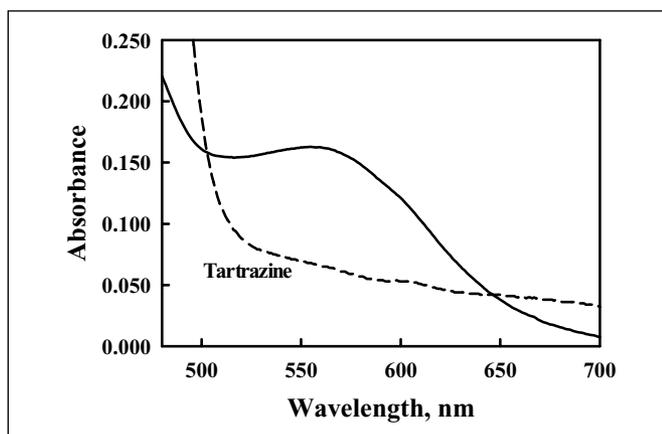
Spoilage of pickle products characterized by the development of a red-color deposit on cucumber skins has occurred sporadically in the commercial pickled vegetable industry for many years. Unpublished results from our laboratory suggested that the red-colored spoilage involved *L. casei* and tartrazine, which is used as a yellow coloring in pickled cucumber products. Results of our current investigation have shown that a strain of *L. paracasei* isolated over 15 y ago from an outbreak, and a strain of *L. casei* isolated from a recent commercial outbreak, caused the red-colored spoilage in cucumber pickles that contain tartrazine as a yellow coloring. *L. casei* and *L. paracasei* isolates were reisolated from pasteurized red-color spoiled cucumber jars. Together these observations fulfilled Koch's postulates; therefore, these 2 isolates were identified as causative agents of red-colored spoilage.

Although several lactobacilli, including *Lactobacillus plantarum* and *Lactobacillus brevis*, have been isolated from vegetables that have undergone natural lactic acid fermentation, *L. casei* and *L. paracasei* are normally absent from these products. *L. plantarum* normally dominates cucumber fermentations. *L. casei* and *L. paracasei* are Gram-positive facultative anaerobic bacteria commonly found in soil, plant material, the intestinal tract of humans and other animals, and foods such as milk, yogurt, wine, and fermented meats. Though they are present in ripened cheeses as the result of contamination (Peterson and Marshall 1990; Jordan and Cogan 1993; Fitzsimons and others 1999; De Angelis and others 2001), it is generally believed that they have a positive influence in cheese flavor development. *L. casei* has also been isolated from spoiled concentrated and frozen orange juices with vinegary to buttermilk off-odor and flavor (Christensen and Pederson 1958). Additionally, it is considered a common beer spoilage microorganism. The exceptional ability of lactobacilli to grow under somewhat extreme conditions and in the presence of limited energy sources allows them to survive in a variety of foods and generate metabolic products that may be desirable or undesirable as judged by the human taste.

Tartrazine, which is an azo dye, and curcumin are used as a natural yellow coloring in pickled cucumber products. *L. casei* LA1133 and *L. paracasei* LA0471 did not degrade the yellow coloring when



**Figure 4—Absorption at 427 and 560 nm of the *Lactobacillus casei* LA1133 and *Lactobacillus paracasei* LA0471 culture supernatants after utilization of tartrazine. Utilization of tartrazine (A) and formation of an unknown product which absorbs at 560 nm (B) by *L. casei* LA1133 (•) and *L. paracasei* LA0471 (Δ) in modified chemically defined media. Disappearance of the tartrazine from cultures supernatants was calculated from the difference between the initial and final absorbance at 427 nm.**



**Figure 5—Absorption spectrum of *Lactobacillus casei* LA1133 culture supernatant. Spectrum before (---) and after (—) utilization of tartrazine.**

**Table 2—Utilization of tartrazine and development of red spoilage in hamburger dill pickles inoculated with a cocktail of *Lactobacillus casei* LA1133 and *Lactobacillus paracasei* LA0471**

Treatment	Growth (+/-)	% of tartrazine utilized	Red color (+/-)
A:1	+	77.9 ± 0.1	+
A:2	+	20.5 ± 0.5	+
A:3	-	0.00 ± 0.1	-
A:4	-	0.00 ± 0.1	-
A:5	-	0.00 ± 0.0	-
B:6	-	10.6 ± 0.1	-
B:7	-	13.5 ± 0.0	-

turmeric was substituted for tartrazine in pasteurized pickle samples. However, utilization of turmeric instead of tartrazine as a coloring agent does not represent an ideal solution for the industry, due to the greater instability of the nonazo dye.

The 2 isolated cultures that were shown to cause the red pigmented spoilage were compared for their ability to grow and utilize tartrazine in jars of pasteurized cucumbers with an initial equilibrated pH of  $5.5 \pm 0.2$  (Figure 2). Though *L. paracasei* LA0471 grew slower than the *L. casei* isolate, it utilized more tartrazine faster than *L. casei*. None of the isolates were capable of utilizing tartrazine when the cucumber jars pH was 2.0 or lower. Although extremely acidic environments may prevent formation of red-colored cucumbers, this solution jeopardizes the suitability of the product for consumption.

Presumably the red color deposited on the cucumber pickles is a product of tartrazine utilization. However, no red precipitate was observed to form on the bottom of jars, which suggests that the red compound may be soluble, but binds to the cucumber surface. When tartrazine was added to culture medium in the absence of cucumbers it was utilized and the culture medium developed a pink color, which did not form in the absence of the tartrazine. There was a small increase in the absorption of the centrifuged culture supernatant at wavelengths longer than the absorption peak of the tartrazine, which is presumed to be a metabolic product. Identification of tartrazine utilization products is the subject of ongoing research.

Utilization of tartrazine was prevented with as little as 0.057% benzoic acid in hamburger dill pickle jars, in which acid and salt were also reduced by 20% compared to the commercial concentrations. Development of red spoilage was prevented by 0.1% sorbic acid only if the concentrations of acid, salt, and calcium chloride were raised to levels that were the maximum practical concentrations for this type of product. These results indicate that addition of sodium benzoate would be a practical method to prevent development of the red pigment spoilage in hamburger dill pickles. Addition of the preservative represents a more suitable solution for the industry as compared to pasteurization. Pasteurization of products packed in bulk is not a cost effective solution for the industry.

The observation that lactobacilli utilize an azo dye is interesting in that removal of azo dyes from waste-water streams is a problem in the textile industry. Considerable effort has been made to identify microorganisms capable of degrading azo dyes in these waste

streams (Sponza and Işik 2002; dos Santos and others 2005). If food grade lactobacilli could be identified that could degrade a range of these dyes, they might be organisms of choice for waste treatment applications.

## Conclusions

Isolates identified as *Lactobacillus casei* (ATCC 393) and *Lactobacillus paracasei* and the azo dye and coloring agent tartrazine were identified as the causative agents of red-color spoilage in cucumber pickles. Substitution of tartrazine with the nonazo dye turmeric and the addition of sodium benzoate to the brine cover solution may prevent the development of red-color spoilage in cucumbers.

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