

# Effects of Prefrying Times on the Nutritive Value of Canned Tilapia Meat

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Tilapia meat was placed in the fryer filled with frying oil preheated to 180 °C. The frying times were 2, 5, 10, 15, or 20 min. The fried meat was then packed in 209 × 409 cans, hermetically sealed, and retorted. As the frying time increased beyond 10 min, available lysine and in vitro protein digestibility of the meat decreased significantly ( $P < 0.05$ ) in fried tilapia meat. Rats on diets of tilapia meat fried for 15 or 20 min containing 10% protein had poor growth and lower net protein utilization (NPU) ( $P < 0.05$ ) than that of rats on diets of tilapia meat fried for 2 or 5 min. Canned tilapia meat prefried for 15 min had higher sensory scores of odor and flavor, whereas the scores of color and texture were high for canned tilapia meat prefried for 10 min.

The value of fish for human nutrition lies in the relatively high protein content, good digestibility, and high biological value of fish proteins (Geiger and Borgstrom, 1962).

Tilapia are mainly lacustrine fish that are well adapted to enclosed waters. They exhibit many qualities that suit them for culture, including resistance to handling and disease, efficient conversion of low-protein diets, ease of breeding, and high palatability; thus, they are an important human protein source in many tropical and subtropic countries. In Taiwan, tilapia production has surpassed the production of milkfish, and thus tilapia has become the preeminent cultured fish species, with yearly production exceeding 100 000 tons.

Various processing procedures must be applied to foods for preservation, quality improvement, and consumer convenience and/or to increase their market value.

The purpose of the study was to evaluate the effects of different prefrying times on the nutritional value of canned tilapia meat.

## MATERIALS AND METHODS

**Product Preparation.** Live commercially harvested tilapia were obtained from the local market. Fish weighed between 700 and 900 g each. After the fish were butchered and rinsed with water, the scales, head, tail, viscera, and central bone were discarded. The remaining tilapia meat was cut into pieces 9 × 2.5 × 1.5 cm and fried in soybean oil (President Food Co.), at a meat to oil ratio of 1:4 (weight) at 180 °C. Frying times were 2, 5, 10, 15, or 20 min. Fresh frying oil was used for each variable frying period. The fried tilapia were cooled and packed in 209 × 409 cans. A total of 150 cans were packed for each treatment, and in each can solid meat were placed with sufficient patching to give a net weight of 90 g. Thirty-milliliter portions of seasoning were then placed on top of the meat, leaving a headspace of about 10 mm. The composition of seasoning per liter of water was sugar, 125 g; salt, 25 g; monosodium glutamate, 25 g; soy sauce, 57 mL; rice wine, 12 mL; and vinegar, 8 mL. All cans were sealed immediately after being exhausted for 15 min. The cans were then steam-retorted at 118 °C for 40 min, allowed to cool under running water, stored at room temperature, and opened 4 weeks after processing. The contents of all 150 cans of each variable were drained through a No. 8 mesh screen for 30 min and pooled. Samples were taken from the pooled meat for the moisture determination (AOAC,

1984). The drained meat was then lyophilized for 10 h at pressures below 100  $\mu$ m Hg (FTS Systems Inc., Stone Ridge, NY), ground to a fine powder (30 mesh), and stored at -20 °C for subsequent analysis.

**Chemical Analysis.** Crude protein, fat, and ash were analyzed by the methods given by the AOAC (1984). The amino acid composition was determined by a column chromatographic method using an amino acid analyzer (LKB 4150 Model). The fish samples were hydrolyzed for 24 h at 110 °C with 6 N HCl under vacuum. Available lysine was estimated by the dye-binding method described by Hurrell et al. (1979). The in vitro protein digestibilities of the samples were determined by the method of Satterlee et al. (1982) using a multienzyme mixture of trypsin, chymotrypsin, peptides, and a bacterial protease.

**Diet Preparation.** Freeze-dried canned tilapia meat was added to diets to provide a total protein content of 10%. The proximate composition of raw and canned tilapia meat and the composition of diets are shown in Tables I and II, respectively.

**Feeding Study.** Thirty-six 28-day-old male Sprague-Dawley rats (Yang Ming Medical School, Animal Center, Taipei, ROC) were used in this study. Six groups of six each animals were fed the experimental diets. The animals weighed from 55 to 65 g at the beginning of the experiment. All of the rats were on standard Purina rat chow for 3 days before the experiments started. During the 10-day experimental period, the animals were housed individually and given water and food ad libitum. Ambient temperature was 25 ± 1 °C.

At the termination of the experiment, animal weight and food consumption were measured. Nitrogen intake of rats was thus obtained through the calculation based on the food consumption. Then, the animals were sacrificed, and the carcass was cut into small pieces and dissolved in 500 mL of 25% KOH solution (in 95% ethanol). Each solution was shaken overnight until the carcass was dissolved completely in alcoholic KOH solution. Gravity filtration was employed, and the contents were washed two times with 200 mL of distilled water each time. The filtrate was then diluted to final volume of 2000 mL with distilled water. A 20-mL aliquot was taken for carcass nitrogen determination by the method of the AOAC (1984). The net protein utilization (NPU) was obtained by (carcass N - carcass of protein-free group) × 100/N intake.

Hedonic scale test (Peryam and Pilgrim, 1957) was used for sensory evaluation. A group of 30 graduate students in the Department of Marine Food Science who had taken a sensory evaluation course were selected as the taste panel. Color, odor, flavor, and texture scores were evaluated.

**Statistical Analysis.** All the data were analyzed for statistical significance by analysis of variance. Multiple

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**Table I. Proximate Composition (%) of Canned Tilapia Meat with Different Prefrying Time<sup>a,b</sup>**

frying time, min	moisture	dry basis			
		crude protein	crude lipid	ash	carbohydrate <sup>c</sup>
raw	76.73 ± 0.02 <sup>a</sup>	74.68 ± 0.41 <sup>a</sup>	19.78 ± 1.17 <sup>e</sup>	5.55 ± 0.61 <sup>a</sup>	
2	70.66 ± 0.86 <sup>b</sup>	66.27 ± 0.28 <sup>b</sup>	21.00 ± 0.59 <sup>e</sup>	5.95 ± 0.68 <sup>a</sup>	6.80
5	69.18 ± 0.11 <sup>bc</sup>	64.15 ± 0.21 <sup>c</sup>	23.91 ± 0.63 <sup>d</sup>	5.55 ± 0.22 <sup>a</sup>	6.39
10	64.92 ± 0.40 <sup>cd</sup>	59.42 ± 0.31 <sup>d</sup>	29.06 ± 0.23 <sup>c</sup>	5.53 ± 0.08 <sup>a</sup>	5.99
15	60.17 ± 3.19 <sup>de</sup>	54.52 ± 0.18 <sup>e</sup>	32.91 ± 0.54 <sup>b</sup>	4.92 ± 0.04 <sup>a</sup>	7.65
20	57.33 ± 0.89 <sup>e</sup>	51.59 ± 1.05 <sup>f</sup>	36.36 ± 0.54 <sup>a</sup>	4.97 ± 0.12 <sup>a</sup>	7.08

<sup>a</sup> Mean ± SD. (For each variable, five samples were taken from pooled 150 cans of tilapia meat.) <sup>b</sup> Figures in the same column having different superscripts (a-f) are significantly different ( $P < 0.05$ ). <sup>c</sup> Obtained through subtraction.

**Table II. Composition of the Diets Used in Animal Study<sup>a</sup>**

ingredient	N-free	C2	C5	C10	C15	C20
canned tilapia meat		15.09	15.59	16.84	18.35	19.39
corn oil	7.05	3.88	3.32	2.16	1.01	0.00
corn starch	82.95	71.03	68.14	68.90	68.96	68.86
vitamin mixture <sup>b</sup>	1.00	1.00	1.00	1.00	1.00	1.00
salt mixture <sup>c</sup>	4.00	4.00	4.00	4.00	4.00	4.00
cellulose	5.00	5.00	5.00	5.00	5.00	5.00

<sup>a</sup> C2, C5, C10, C15, C20: canned tilapia meat prefried at 2, 5, 10, 15, and 20 min, respectively. <sup>b</sup> Vitamin mixture (g/100 g of diet): D-biotin 1%, 20.0; vitamin D (200 IU/g), 0.25; folic acid, 1.0; thiamin hydrochloride, 1.5; riboflavin, 1.5; menadione, 1.5; vitamin A concentrate (500 000 IU/g), 2.0; niacin, 5.0; pyridoxine hydrochloride, 1.0; calcium D-pantothenate, 5.0; D,L- $\alpha$ -tocopheryl acetate, 20.0; choline bitartrate, 100.0; vitamin B<sub>12</sub>, 0.02. <sup>c</sup> Salt mixture (g/100 g of mixture): CaCO<sub>3</sub>, 19.56; CaHPO<sub>4</sub>·2H<sub>2</sub>O, 38.60; Na<sub>2</sub>HPO<sub>4</sub>, 17.58; KCl, 19.75; MgSO<sub>4</sub>, 11.60; MnSO<sub>4</sub>·H<sub>2</sub>O, 0.402; CuSO<sub>4</sub>, 0.071; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.084; KIO<sub>3</sub>, 0.028; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.473.

comparisons among means were made with the Duncan's new multiple-range test (Puri and Mullen, 1980).

## RESULTS AND DISCUSSION

Moisture and crude protein of the fish meat decreased as the frying time increased (Table I). The decrease in protein content of tilapia meat with the increased frying time is in agreement with Ponnampalam and Mondy (1983). While the lipid content increased significantly ( $P < 0.05$ ) as the frying time increased, ash and carbohydrate of the fish meat were not affected by the different frying times ( $P > 0.05$ ).

The raw tilapia meat contained negligible carbohydrate, but because of the absorption of sugar from the seasoning, the canned tilapia meat contained about 6–7% carbohydrate (Table I). The loss of volatile components generated during frying (Lee et al., 1973) and some nitrogenous compounds leached from the tissue by the oil may account for the decrease in protein content of tilapia meat as the frying time increased. The carbohydrate added to the fried product as it went into the can was sugar. Heating has been reported to decrease available lysine in the sucrose diet (Westring and Potter, 1984). This loss is most likely due to hydrolysis of the sucrose and subsequent reaction of the resulting reducing monosaccharides with lysine to form biologically unavailable premelanoidins (Finot, 1982). Thus, nonenzymatic browning reactions (Maillard) in which amino acids react with carbohydrate at high temperatures producing brown melanoidins may also be partially responsible for losses of amino acids. Another possible explanation of the loss of nitrogen compounds may lie in the fact that polyunsaturated lipids in the presence of oxygen and heat could produce dicarbonyls that may react with amino acids to form melanoidins or pyrazines (Mauron, 1972).

The amino acid profiles of canned tilapia meat prefried at different times are shown in Table III. Heat damage of proteins results from the destruction of amino acids within a protein, reactions of proteins with nonprotein

**Table III. Amino Acid Profile (g/100 g of Protein) of Canned Tilapia Meat Fried for Different Periods<sup>a,b</sup>**

amino acid	C0	C2	C5	C10	C15	C20
lysine	9.95	9.95	10.01	9.80	9.18	7.79
histidine	2.58	2.41	2.32	2.54	2.54	2.25
arginine	6.88	5.85	5.81	5.48	5.12	5.47
aspartic acid	10.23	10.88	11.40	10.92	11.68	10.28
threonine	4.05	1.99	2.10	2.24	2.17	2.30
serine	3.21	3.04	3.52	3.50	3.69	3.73
glutamic acid	15.11	17.02	17.84	17.83	16.80	17.08
proline	4.93	4.43	4.28	4.07	4.10	4.98
glycine	6.89	5.91	5.89	5.27	6.20	8.45
alanine	7.22	6.72	6.95	6.62	7.25	7.74
cysteine	1.26	1.28	1.24	1.26	1.15	1.08
valine	5.27	6.06	6.18	5.99	5.74	5.34
methionine	2.87	2.17	1.96	2.22	2.50	2.19
isoleucine	4.48	5.13	4.58	5.18	5.20	4.65
leucine	8.16	8.75	8.13	8.80	8.70	8.28
tyrosine	2.93	2.92	2.77	3.08	3.26	3.13
phenylalanine	3.97	5.50	4.99	5.19	4.70	5.26

<sup>a</sup> C0, C2, C5, C10, C15, C20: canned tilapia meat prefried at 0, 2, 5, 10, 15, and 20 min, respectively. <sup>b</sup> Sample was taken from pooled 150 cans of tilapia meat in each variable.

**Table IV. Available Lysine Content and in Vitro Protein Digestibility of Canned Tilapia Meat Prefried for Different Periods<sup>a,b</sup>**

frying time, min	available Lys, g/100 g protein	Lys, availability, %	in vitro protein, digestibility, %
0	9.38 ± 0.31 <sup>a</sup>	94.22 ± 2.61 <sup>a</sup>	87.93 ± 1.96 <sup>a</sup>
2	9.12 ± 0.39 <sup>a</sup>	91.64 ± 3.33 <sup>a</sup>	86.17 ± 1.72 <sup>ab</sup>
5	8.96 ± 0.06 <sup>a</sup>	89.54 ± 0.49 <sup>a</sup>	84.11 ± 2.17 <sup>abc</sup>
10	8.53 ± 0.45 <sup>ab</sup>	87.02 ± 3.37 <sup>ab</sup>	83.93 ± 1.95 <sup>abc</sup>
15	7.94 ± 0.23 <sup>b</sup>	86.50 ± 2.14 <sup>b</sup>	81.88 ± 2.03 <sup>bc</sup>
20	6.68 ± 0.37 <sup>c</sup>	85.72 ± 4.08 <sup>b</sup>	80.09 ± 2.58 <sup>c</sup>

<sup>a</sup> Mean ± S.D. (For each variable, five samples were taken from pooled 150 cans of tilapia meat.) <sup>b</sup> Figures in the same column having different superscripts (a-c) are significantly different ( $P < 0.05$ ).

components in a food, or inter- and intraprotein reactions in the presence or absence of oxygen (Carpenter et al., 1962). In the present experiment, lysine, histidine, arginine, methionine, and threonine contents of canned tilapia meat were decreased as the frying process was employed.

Available lysine content and in vitro protein digestibility of canned tilapia meat with different frying times are shown in Table IV. Both available lysine and protein digestibility decreased as frying time increased. One of the most important factors associated with the loss of the nutritional value of proteins is the formation of new linkages within and between protein molecules (Ford, 1983). These reactions cause restructuring of protein molecules, indirectly affect the nutritional value of the protein by changing the rate or extent of enzymatic hydrolysis, and in turn impair digestibility. These reactions affect the availability of amino acids at the specific site of enzymatic cleavage as well as the availability of adjacent amino acids. In our study, lysine availability decreased

**Table V. Weight Gain, Food Intake, and NPU of Rats Fed Otherwise Adequate but Protein-Free Diets or the Same Diet Containing 10% Protein from Canned Tilapia Meat Prefried at Different Times as the Sole Source of Dietary Protein<sup>a-c</sup>**

treatment	no. of rats	wt gain, g	food intake, g	NPU, %
protein-free	6	-8.68 ± 4.19 <sup>d</sup>	43.52 ± 4.97 <sup>b</sup>	
C2	6	36.57 ± 9.09 <sup>ab</sup>	81.42 ± 15.78 <sup>a</sup>	77.98 ± 8.39 <sup>a</sup>
C5	6	40.18 ± 8.70 <sup>a</sup>	95.55 ± 18.83 <sup>a</sup>	69.52 ± 4.17 <sup>ab</sup>
C10	6	35.17 ± 5.23 <sup>ab</sup>	85.82 ± 10.27 <sup>a</sup>	61.62 ± 8.35 <sup>b</sup>
C15	6	20.47 ± 6.51 <sup>c</sup>	74.53 ± 13.81 <sup>a</sup>	49.40 ± 6.05 <sup>c</sup>
C20	6	19.63 ± 7.22 <sup>c</sup>	75.70 ± 14.05 <sup>a</sup>	46.27 ± 5.61 <sup>c</sup>

<sup>a</sup> Mean ± SD ( $n = 6$ ). <sup>b</sup> Figures in the same column having different superscripts are significantly different ( $P < 0.05$ ). <sup>c</sup> C2, C5, C10, C15, C20: canned tilapia meat prefried at 2, 5, 10, 15, and 20 min, respectively.

**Table VI. Sensory Evaluation of Canned Tilapia Meat with Different Prefrying Times<sup>a-d</sup>**

treatment	color	odor	flavor	texture
C2	3.0 ± 1.1 <sup>d</sup>	2.2 ± 1.1 <sup>d</sup>	2.4 ± 1.0 <sup>d</sup>	2.4 ± 1.2 <sup>c</sup>
C5	6.2 ± 1.1 <sup>b</sup>	5.2 ± 1.3 <sup>c</sup>	5.6 ± 1.5 <sup>c</sup>	5.1 ± 1.4 <sup>b</sup>
C10	7.8 ± 1.4 <sup>a</sup>	6.5 ± 1.7 <sup>b</sup>	6.0 ± 1.4 <sup>bc</sup>	6.2 ± 1.0 <sup>a</sup>
C15	6.8 ± 1.8 <sup>b</sup>	7.8 ± 1.1 <sup>a</sup>	7.4 ± 1.3 <sup>a</sup>	6.8 ± 1.4 <sup>a</sup>
C20	5.0 ± 1.6 <sup>c</sup>	7.2 ± 1.3 <sup>ab</sup>	6.6 ± 1.3 <sup>ab</sup>	5.3 ± 1.5 <sup>b</sup>

<sup>a</sup> Mean ± SD ( $n = 30$ ). <sup>b</sup> Figures in the same column having different superscripts are significantly different ( $P < 0.05$ ). <sup>c</sup> C0, C2, C5, C10, C15, C20: canned tilapia meat prefried at 0, 2, 5, 10, 15, and 20 min, respectively. <sup>d</sup> Hedonic scale: 9, like extremely; 5, neither like nor dislike; 1, dislike extremely.

as the frying time increased and one should note that when the frying time was beyond 15 min, the available lysine content of tilapia meat decreased significantly ( $P < 0.05$ ) (Table IV).

The performance and net protein utilization of rats are presented in Table V. The weight gain and NPU value were highest for rats consuming diets in which the protein source was from tilapia meat prefried for 5 min. These values declined as the frying time increased. The available lysine and protein digestibility of tilapia meat all decreased when the meat was prefried at longer times (Table IV). Presumably the rats became malnourished with a diet containing canned tilapia meat fried for longer times.

During the frying process, many complex changes may have taken place. As the tilapia meat enters hot oil, oxygen is introduced into the oil, which leads to oxidation. As the meat absorbs frying fat, moisture is released from the fried meat (Table I), resulting in hydrolysis of triglyceride to form free fatty acids, diglycerides, monoglycerides, and glycerol (Buziassy and Nawar, 1968; Noble et al., 1967; Lillard, 1983). Heating the oil to high frying temperatures leads to formation of polymers as the reaction products condense. As these reactions take place, the sensory and nutritional qualities of the fat change and the oil can no longer be used to produce high-quality foods (Landers and Rathman, 1981; Paulose and Chang, 1973; Witchwoot et al., 1981). In the present investigation, the frying oil was replaced with fresh oil after each frying process, avoiding the possible detrimental effect of the deteriorated oil on the tilapia meat so that the changes of nutritive value of canned tilapia meat would be solely from the prefrying process.

The sensory evaluation of canned tilapia meat with different frying times is presented in Table VI. The score for color was high in canned tilapia meat prefried for 10 min followed by that prefried for 15 or 5 min. The score for texture was high for canned tilapia meat prefried for 10 and 15 min followed by that prefried for 5 or 20 min. The scores of odor and flavor were high in canned tilapia meat prefried for 15 min followed by that prefried for 10 or 20 min.

In conclusion, the sensory evaluation results indicated that canned tilapia meat prefried for 10 min had the highest scores in color and texture. The scores of odor and

flavor of canned tilapia meat prefried for 10 min were lower than those of meat prefried for 15 min. However, the results also demonstrated that increasing the time of the deep-fat prefried process resulted in a decrease of available lysine and in vitro protein digestibility. The growth and net protein utilization of rats decreased when rats received diets containing canned tilapia meat prefried more than 10 min. The findings reported here are of value to nutritionists and food scientists concerned about the optimum prefrying procedure of the canned tilapia meat.

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**Registry No.** L-Lysine, 56-87-1; L-histidine, 71-00-1; L-arginine, 74-79-3; L-methionine, 63-68-3; L-threonine, 72-19-5.

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## Trichothecene Toxin Production by Strains of *Gibberella pulicaris* (*Fusarium sambucinum*) in Liquid Culture and in Potato Tubers

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Fifteen strains of *Gibberella pulicaris* (asexual stage: *Fusarium sambucinum* or *Fusarium sulphureum*) isolated from dry-rotted potato (*Solanum tuberosum*) tubers were tested for trichothecene toxin production in liquid culture and in potato tubers, for pathogenicity on potato tubers, and for ability to form sexual crosses. Fourteen strains were sexually fertile and accumulated 4,15-diacetoxyscirpenol in liquid culture (up to 47  $\mu\text{g}$  of toxin/mg dry weight fungal mass). Most strains were able to rot tuber slices of potato cultivar Russet Burbank and produced 15-monoacetoxyscirpenol and 4,15-diacetoxyscirpenol as well as other minor trichothecenes in infected tubers (up to 5  $\mu\text{g}$  of toxin/g rot fresh weight). These results indicate that production of trichothecene toxins is a common trait of *G. pulicaris* strains isolated from potato tuber dry-rot and that trichothecenes may occur in potatoes naturally infected with this fungus in the field or in storage.

Diacetoxyscirpenol and other trichothecene toxins are produced by a number of *Fusarium* species, including *Gibberella pulicaris* (Fries) Sacc. (asexual stage: *Fusarium sambucinum* Fuckel or *Fusarium sulphureum* Schlecht). The trichothecenes are a closely related group of sesquiterpenes that are potent eukaryotic protein synthesis inhibitors and are associated with a variety of mycotoxicoses in humans and animals (Mararas et al., 1984). *G. pulicaris* is a major cause worldwide of dry-rot of potato tubers (Boyd, 1972; Jeffries et al., 1984). Trichothecene-producing strains of *G. pulicaris* have been isolated as dominant fungal species from rotted potato tubers collected in Germany (Siegfried and Langerfeld, 1978), in France (Lafont et al., 1983), in Poland (Latus et al., 1987), and in a high-incidence area of human esophageal cancer in Iran (Steyn et al., 1978). Despite the widespread occurrence of *G. pulicaris* rot of potato tubers and the documented toxigenicity of strains of *G. pulicaris* isolated from a wide variety of habitats (Mararas et al., 1984; Desjardins and Beremand, 1987), there has been limited study of the ability of *G. pulicaris* to produce trichothecenes in potato tubers. In France, Lafont and co-workers (1983) found several trichothecene toxins in potato tubers naturally and experimentally infected with *G. pulicaris*. In Canada, El-Banna and co-workers (1984) found low concentrations of trichothecenes in potato tubers infected with a strain of *G. pulicaris* that had originally been isolated from potato. We report here the results of a study of 15 single-spored strains of *G. pulicaris* isolated from potato tubers

from several widely separated geographic locations, including their trichothecene toxin production in liquid cultures and in potato tubers, their pathogenicity on potato tubers, and their sexual fertility.

### MATERIALS AND METHODS

**Source of Strains and Culture Conditions.** Strains with the prefix R were identified and supplied by P. Nelson, Fusarium Research Center, The Pennsylvania State University. Strains DAOM 192963, DAOM 192966, and DAOM 196035 were from G. Neish, Biosystematics Research Institute, Agriculture Canada. Strains NRRL 13700 and NRRL 13712 were from A. Murphy, Agriculture Canada. Strains KF-728 and KF-735 were from P. Golinski, Agricultural University of Poznan, Poland. Strain NRRL 13711 was from the author's laboratory, NRRL 13500 from R. Caldwell, University of Wisconsin, and NRRL 13707 from S. Leach, University of Maine. Information on strain identification and habitat was provided by the investigator who supplied the strain. Strain NRRL 13711 was identified by M. Beremand, Northern Regional Research Center. All cultures were reisolated from single spores prior to this study, and the NRRL numbers refer to single-spore strains. Cultures were routinely grown on V-8 juice agar slants (Stevens, 1974) on an alternating 12 h/25 °C light and 12 h/20 °C dark schedule. Cultural characteristics such as pigmentation were based on 10-14-day-old cultures on potato dextrose agar (Nelson et al., 1983). For long-term storage, strains were maintained on V-8 agar slants at 4 °C and as lyophilized conidial suspensions in the Northern Regional Research Center collection (NRRL prefix), Peoria, IL. For all experiments reported here, fresh transfers of the strains were obtained from stock cultures stored at 4 °C.

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