**ABSTRACT** *Listeria monocytogenes* is a foodborne pathogen that causes high rates of hospitalization and mortality in people infected. Contamination of fresh, ready to eat produce by this pathogen is especially troubling because of the ability of this bacterium to grow on produce under refrigeration temperatures. In this study, we created a library of over 8,000 plant phyllosphere-associated bacteria and screened them for the ability to inhibit the growth of *L. monocytogenes* in an *in vitro* fluorescence-based assay. One isolate, later identified as *Bacillus amyloliquefaciens* ALB65, was able to inhibit the fluorescence of *L. monocytogenes* by >30-fold *in vitro*. *B. amyloliquefaciens* ALB65 was also able to grow, persist, and reduce the growth of *L. monocytogenes* by ≥1.5 log CFU on cantaloupe melon rinds inoculated with 5 × 10³ CFU at 30°C and was able to completely inhibit its growth at temperatures below 8°C. DNA sequence analysis of the *B. amyloliquefaciens* ALB65 genome revealed six gene clusters that are predicted to encode genes for antibiotic production; however, no plant or human virulence factors were identified. These data suggest that *B. amyloliquefaciens* ALB65 is an effective and safe biological control agent for the reduction of *L. monocytogenes* growth on intact cantaloupe melons and possibly other types of produce.

**IMPORTANCE** *Listeria monocytogenes* is estimated by the Centers for Disease Control and Prevention and the U.S. Food and Drug Administration to cause disease in approximately 1,600 to 2,500 people in the United States every year. The largest known outbreak of listeriosis in the United States was associated with intact cantaloupe melons in 2011, resulting in 147 hospitalizations and 33 deaths. In this study, we demonstrated that *Bacillus amyloliquefaciens* ALB65 is an effective biological control agent for the reduction of *L. monocytogenes* growth on intact cantaloupe melons under both pre- and postharvest conditions. Furthermore, we demonstrated that *B. amyloliquefaciens* ALB65 can completely inhibit the growth of *L. monocytogenes* during cold storage (<8°C).

**KEYWORDS** biocontrol, cantaloupe, secondary metabolites, *Listeria monocytogenes*, *Bacillus amyloliquefaciens*

*Listeria monocytogenes* has been a problematic foodborne pathogen of public health importance for many years (1). Due to its ubiquitous nature, preventing it from entering into food production environments has been a challenge (1–4). Especially troubling is the fact that *L. monocytogenes* can grow at refrigeration temperatures (0 to 5°C), allowing it to grow on produce held under cold storage (2, 5). *L. monocytogenes* infections result in high hospitalization rates and the U.S. Food and Drug Administration (FDA) estimates the mortality rate to be as high as 20 to 30% (2). The Centers for Disease Control and Prevention (CDC) (6) and the FDA (7) estimate that approximately 1,600 to 2,500 people suffer from listeriosis in the United States every year, causing an
economic burden of approximately $2.6 billion, making it the third most costly foodborne illness in the United States (7).

Since 2011, several multistate foodborne listeriosis outbreaks have been associated with milk, soft cheeses, deli meats, and fresh produce (8). Especially concerning is the increased rate of contamination of fresh, ready-to-eat produce, including cantaloupe melons (2, 8, 9). In 2011, the largest outbreak of listeriosis in the United States was associated with uncut cantaloupe melons and resulted in 147 illnesses and 33 deaths in 28 states (10). Fresh produce can become contaminated with *L. monocytogenes* during the pre- and postharvest stages via direct or indirect contact with infected animals, contaminated soil, water, equipment, and human processors (2, 10). The prevention of *L. monocytogenes* contamination of fresh, ready-to-eat fruit and vegetables is a serious food safety challenge, and a method to prevent *L. monocytogenes* from colonizing produce at both the pre- and postharvest stages would be advantageous to both producers and consumers.

Biological control is a bioeffector method of pest control and has been used to control the growth of insects (11), invasive weeds (12, 13), plant pathogens (14–16), and other unwanted organisms. Several studies have proposed the use of lytic bacteriophages for the biological control of *L. monocytogenes* because of their ability to infect and lyse this bacterium (1, 17–21). Virulent bacteriophages have been used as biological control agents in many ready-to-eat foods such as milk, cheese, hot dogs (20), and raw meat (17, 19). Application of mixtures of the phages LM-103 and LMP-102 with the bacteriocin nisin significantly reduced *L. monocytogenes* populations on sliced honeydew melons and apples (21). Bacteria have also been used as biological control agents. For example, Leverentz et al. (22) reported the use of bacteria that naturally occur on the surfaces of apples to inhibit the growth of *L. monocytogenes* on fresh-cut apples. Others have shown that bacteriocinogenic lactic acid bacteria, such as *Leuconostoc citreum* MB1 and *Enterococcus mundtii* CRL35, were able to delay *L. monocytogenes* growth in milk at refrigeration temperatures (23) and during sausage fermentation (24), respectively. In addition, Sharma et al. (25) showed that *Azotobacter chroococcum*, *Bacillus megaterium*, and *Pseudomonas fluorescens* were effective as biopesticides for the reduction of *L. monocytogenes* in the rhizosphere of pigeon pea plants (*Cajanus cajan*). However, bacterial biocontrol agents have rarely been examined for the ability to prevent *L. monocytogenes* from colonizing and persisting on the surfaces of intact, unprocessed fruits such as cantaloupe melons. Here, we used a novel, high-throughput in vitro screening methodology to identify plant phyllosphere associated bacteria that can be used as biological control agents to prevent/reduce the growth of *L. monocytogenes* on cantaloupe melons.

**RESULTS**

**Identification of PPAB capable of inhibiting the growth of *L. monocytogenes* in vitro.** We created and screened a plant phyllosphere associated bacterial library for the ability to inhibit the growth of *L. monocytogenes* RM2387-pNF8 in an in vitro fluorescence assay. Twenty PPAB isolates, named ALB65 to ALB84 for anti-*Listeria* bacteria, were identified that could inhibit the fluorescence of *L. monocytogenes* RM2387-pNF8 by 13.6- to 31.3-fold after 48 h, compared to the control (data not shown). We also assayed 18-h cell-free culture supernatants from these bacteria for the ability to inhibit the growth of this bacterium and found that 7 of the 20 isolates were able to inhibit the fluorescence of *L. monocytogenes* RM2387-pNF8 by 10.4- to 22.2-fold after 48 h (data not shown). To identify these isolates, we sequenced their 16S rRNA genes and performed BLAST analysis against the NCBI nucleotide database that revealed all of them were ~99% identical to *Bacillus* spp. Isolate ALB65 produced the greatest fluorescence inhibition of *L. monocytogenes* RM2387-pNF8 in vitro (Table 1) and was selected for further analysis.

Genomic DNA sequence analysis of ALB65 revealed that it was 98.72% identical to the plant-associated bacterium *Bacillus amyloliquefaciens* FZB42 and 95.49% identical to the soil-associated bacterium *B. amyloliquefaciens* DSM7 (26, 27). To confirm the
ability of *B. amyloliquefaciens* ALB65 to inhibit the growth of multiple *L. monocytogenes* outbreak strains, we performed disk diffusion assays using cell-free culture supernatant from *B. amyloliquefaciens* ALB65 against *L. monocytogenes* RM2387-pNF8 and 10 additional *L. monocytogenes* outbreak strains, all of which produced zones of inhibition of 10 to 14 mm (Table 2).

**Growth and persistence of *B. amyloliquefaciens* ALB65 on cantaloupe melon rinds.** We examined the ability of *B. amyloliquefaciens* ALB65 to grow and persist on the rinds of cantaloupe melons. Growth assays showed that when *B. amyloliquefaciens* ALB65 was inoculated onto cantaloupe rinds at approximately $1 \times 10^4 \text{ CFU rind}^{-1}$, it was able to multiply to $>1 \times 10^6 \text{ CFU rind}^{-1}$ after 48 h at 30°C (Fig. 1A). We also examined the ability of *B. amyloliquefaciens* ALB65 to persist on cantaloupe rinds kept under refrigeration temperatures. When *B. amyloliquefaciens* ALB65 was inoculated at $1 \times 10^7 \text{ CFU rind}^{-1}$ and incubated at 8°C, we observed a significant ($P < 0.05$) decrease of $\sim 2$ logs after 6 days. However, after that time point the number of CFU rind$^{-1}$ did not decrease significantly throughout the 9-day experiment (Fig. 1B). To determine whether *B. amyloliquefaciens* ALB65 was able to persist on the surfaces of preharvested, immature cantaloupe melons still attached to the plant, we sprayed greenhouse-grown melons with *B. amyloliquefaciens* ALB65 and quantified the number of CFU on the melon rinds. After 1 day, the number of *B. amyloliquefaciens* ALB65 was approximately $1 \times 10^5 \text{ CFU rind}^{-1}$ and did not change significantly ($P > 0.05$) throughout the 6-day experiment (Fig. 1C).

**Inhibition of *L. monocytogenes* growth on cantaloupe melon rinds.** We examined the ability of *B. amyloliquefaciens* ALB65 to inhibit the growth of *L. monocytogenes* RM15995, an isolate from the 2011 U.S. multistate cantaloupe outbreak (28), on cantaloupe rinds under conditions that simulated both pre- and postharvest contamination. We coated cantaloupes with *B. amyloliquefaciens* ALB65 or phosphate-buffered saline (PBS [control]), inoculated them with approximately $5 \times 10^3 \text{ CFU L. monocytogenes} \text{ RM15995 rind}^{-1}$, and incubated them at 30°C. *L. monocytogenes* RM15995 grew rapidly on the control rinds, reaching $>2.9 \times 10^6 \text{ CFU rind}^{-1}$ in 24 h; however, on the

### TABLE 1 Fluorescence inhibition of *L. monocytogenes* RM2387 pNF8 by *B. amyloliquefaciens* ALB65

| Sample                                      | Avg RFU (SD) | Fold difference | P     |
|---------------------------------------------|--------------|-----------------|-------|
| *B. amyloliquefaciens* ALB65 culture$^b$    | 5,014.5 (244.0) | 30.2            | <0.001|
| *B. amyloliquefaciens* ALB65 supernatant$^c$| 6,838.3 (382.1) | 22.2            | <0.001|
| BHI control$^d$                             | 151,811.3 (30,625.6) | NA$^e$         | NA    |

$^a$RFU, relative fluorescence units.

$^b$*B. amyloliquefaciens* ALB65 culture, an 18-h culture of *B. amyloliquefaciens* ALB65.

$^c$*B. amyloliquefaciens* ALB65 supernatant, cell-free supernatant from an 18-h culture of *B. amyloliquefaciens* ALB65.

$^d$BHI control, brain heart infusion broth.

$^e$NA, not applicable.

### TABLE 2 Inhibitory activity of *B. amyloliquefaciens* ALB65 cell-free culture supernatant against *L. monocytogenes* outbreak strains

| Strain                  | Serotype | Outbreak source, yr | Zone size (mm)$^a$ | Reference |
|-------------------------|----------|---------------------|--------------------|-----------|
| RM2387-pNF8             | 4b       | None                | 10.0               | 50        |
| RM20667                 | 4b       | Caramel apple, 2014 | 13.0               | 54        |
| RM20669                 | 4b       | Stone fruit, 2014   | 13.0               | 55        |
| RM15995                 | 1/2a     | Cantaloupe, 2011    | 10.0               | 28        |
| RM15996                 | 1/2b     | Cantaloupe, 2011    | 10.0               | 28        |
| RM15997                 | 1/2a     | Cantaloupe, 2011    | 12.0               | 28        |
| RM3180                  | 4b       | Meat, 1998–1999     | 13.5               | 56        |
| RM3177                  | 4b       | Paté, 1987–1989     | 14.0               | 57        |
| RM2199                  | 4b       | Cheese, 1985        | 14.0               | 58        |
| RM3176                  | 4b       | Coleslaw, 1981      | 13.0               | 59        |
| RM15994                 | 4b       | Produce, 1979       | 13.0               | 60        |

$^a$Zone sizes are shown as mean values from three independent experiments.
**B. amyloliquefaciens** ALB65-treated rinds, **L. monocytogenes** only reached $4.5 \times 10^4$ CFU rind$^{-1}$, an $-1.5$-log CFU reduction in growth (Fig. 2A). We also examined **B. amyloliquefaciens** ALB65 for the ability to inhibit the growth of **L. monocytogenes** under simulated postharvest cold-storage conditions. We applied **B. amyloliquefaciens** ALB65 to cantaloupe rinds, inoculated them with **L. monocytogenes** as described above, and incubated them at 8°C. After 6 days **L. monocytogenes** grew to approximately $8.3 \times 10^4$ CFU on the PBS control rinds; however, on the **B. amyloliquefaciens** ALB65 treated rinds, we did not observe any increase in the number of **L. monocytogenes** CFU rind$^{-1}$ (Fig. 2B).

**Predicted secondary metabolite gene clusters in the **B. amyloliquefaciens** ALB65 genome.** We sequenced the genome of **B. amyloliquefaciens** ALB65 using a combination of Pacific Biosciences (PacBio) RS II and Illumina MiSeq platforms (27). *In silico* analysis of the **B. amyloliquefaciens** ALB65 genome via antiSMASH 4.0 (29) revealed six gene clusters, representing ~5.7% of the genome, that were predicted to encode genes for the biosynthesis of the antibiotic compounds macrolactin, difficidin, bacillaene, bacilysin, bacillibactin, and amylocyclicin (Table 3). All the gene clusters shared 90 to 100% similarity with the corresponding gene clusters in **B. amyloliquefaciens** FZB42, except for the bacillibactin gene cluster that shared 100% similarity to that found in **B. subtilis** subsp. **subtilis** 168.

**DISCUSSION**

The contamination of fresh, ready to eat produce by human-pathogenic bacteria continues to be a challenge for the food industry, government agencies and ultimately the consumer (30). Several methods to reduce/eliminate pathogens from produce have been implemented, the most commonly used methods employ physical or chemical treatments (31, 32). A third approach to preventing/reducing pathogen contamination...
of produce is biocontrol, a bioeffector method of controlling pests using other living organisms (33). In order to be an effective biocontrol agent, the organism must be able to grow and persist on the surface of the target produce (31). We demonstrated that B. amyloliquefaciens ALB65 was able to grow and persist on cantaloupe melons under both pre- and postharvest conditions, as well as during cold storage. In addition, biological control agents must not induce early rot of the produce, impart off colors or smells, or produce virulence factors against the host plant or humans (34). B. amyloliquefaciens ALB65 did not induce early rot, nor did it impart off colors or smells to the cantaloupes after treatment and genome sequence analysis did not detect any virulence associated genes (data not shown). If applied to plants preharvest, biological control agents must not inhibit plant growth or fruit production. When we tested B. amyloliquefaciens ALB65 for its effects on cantaloupe plant growth, we observed that treated cantaloupes grew twice as fast in length as untreated cantaloupes and produced fruit significantly earlier as well (data not shown). This finding is consistent with previously published studies that have shown that B. amyloliquefaciens FZB42 enhances plant growth (26, 35). Lastly, effective biocontrol agents must be able to inhibit the target pathogen’s growth on produce (34). We demonstrated that B. amyloliquefaciens ALB65 could significantly reduce the growth of L. monocytogenes on cantaloupe rinds.

![Graph A](image1.png)

**FIG 2** B. amyloliquefaciens ALB65 significantly reduced the growth of L. monocytogenes on cantaloupe rinds at 30°C after 24 h (A) and at 8°C after 6 days (B). Data represent mean values and standard deviations from at least three independent experiments in which each melon rind was considered as a replicate.

| Cluster | Antibiotic | Genes present | Size (kbp) (genome location) | Target | Reference(s) |
|---------|------------|---------------|-----------------------------|--------|--------------|
| 1       | Amylocyclicin | acnA to acnF | 4.2 (3132789–3137021) | Cell membrane | 40 |
| 2       | Macrolactin | pks2A to pks2I, pdhA | 54.8 (1460734–1515575) | Protein synthesis | 36, 61 |
| 3       | Difficidin | difA to difO | 69.6 (2367149–2436796) | Protein synthesis | 36, 61 |
| 4       | Bacillaene | baeB to baeE, baeG to baeJ, baeLMN, baeR, baeS, acpK | 72.5 (1772608–1845106) | Protein synthesis | 36, 61 |
| 5       | Bacilysin | bacA to bacE | 4.7 (3711152–3715899) | Cell wall synthesis | 37, 62 |
| 6       | Bacillibactin | ald, yukJ, mbh, dhbABC, dhbE, dhbF, besA, yuiH, bioYB, hisP, pepA | 19.5 (3107309–3126774) | Iron sequestration | 39, 63 |

**TABLE 3** Predicted antibiotic gene clusters in the B. amyloliquefaciens ALB65 genome
at 30°C (P < 0.05) and completely inhibit the growth of this pathogen on cantaloupe rinds at temperatures below 8°C.

It is unclear how *B. amyloliquefaciens* ALB65 inhibits the growth of *L. monocytogenes* on produce. However, genome sequence analysis identified multiple gene clusters that are predicted to produce antibacterial compounds, including macroactin, difficidin, bacillaene, bacilysin, bacillibactin, and amylocyclicin. Macrolactin, difficidin, and bacillaene are polyketide antibiotics that act by inhibiting protein synthesis and have been shown to be active against both Gram-positive and -negative bacteria, including *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli*, and *Ralstonia solanacearum* (36). Bacilysin is a dipeptide antibiotic composed of anticapsin and alanine moieties and has been shown to inhibit cell wall biosynthesis in both Gram-positive and -negative bacteria, including *Erwinia amylovora* (37) and *Staphylococcus aureus* (38). Bacillibactin is a lipopeptide siderophore that sequesters iron, making it unavailable for other microorganisms and thus inhibits their growth (39). Finally, amylocyclicin is a circular bacteriocin produced by several species of *Bacillus* that are most effective against Gram-positive bacteria by forming pores in their cell membranes (40, 41). Recently, amylocyclicin was purified and shown to have strong inhibitory activity against *L. monocytogenes* and *B. cereus* (41).

Previously, strains of *B. amyloliquefaciens* have been shown to exert biocontrol activities against plant-pathogenic fungi and bacteria (15, 42), including brown rot of stone fruit caused by *Monilinia fructigena* (42), root rot of ginseng caused by *Cylindrocarpon destructans* (43), root and stem rot of soybeans caused by *Phytophthora sojae* (44), bacterial canker disease caused by *Clavibacter michiganensis* (45), tomato wilt caused by *Ralstonia solanacearum* (46), and crown gall caused by *Agrobacterium tumefaciens* (47). Strains of *B. amyloliquefaciens* are also commercially used as safe and effective biofertilizers for plant growth promotion in multiple crops (48, 49). In the present study, we demonstrated that *B. amyloliquefaciens* ALB65 is also an effective, food-grade, biological control agent for the reduction of *L. monocytogenes* growth on cantaloupe melons during both the pre- and postharvest periods.

**MATERIALS AND METHODS**

**Isolation of PPAB.** To isolate plant phyllosphere-associated bacteria (PPAB), we used a modification of the protocol described by McGarvey et al. (34). Briefly, we obtained several different types of plant material, including almond drupes, alfalfa, strawberries, cranberries, onions, red lettuce, iceberg lettuce, kale, cucumbers, celery, cabbage, cherries, green beans, jalapenos, okra, ginger, broccoli, cauliflower, and grapes from local grocery stores. Portions (50 g) of each type of produce were suspended in 100 ml of PBS with 0.01% Tween 80 (PBS + T) and 5-mm glass beads and then shaken at 300 rpm at 25°C for 60 min. The liquid was decanted and plated onto different types of bacteriological culture media, including de Mann, Rogosa, and Sharp (MRS; Difco, Sparks, MD), tryptic soy broth (TSB; Oxoid, Basingstoke, Hampshire, England), Reasoner’s 2A (R2A; Remel, Lenexa, KS), pseudomonas agar base (PAB; Oxoid, Basingstoke, Hants, UK), and pseudomonas isolation agar (PIA; Oxoid), followed by incubation at 30°C for 24 h. Well-isolated colonies with sharp edges and diameters of ≥0.5 mm were transferred to 96-well plates containing tryptic soy broth (TSB; Oxoid) with 10% glycerol using a QPix420 automated picking robot (Molecular Devices, San Jose, CA) and stored at −80°C until needed. In total, 8,736 PPAB isolates were collected.

**Inhibition of *L. monocytogenes* growth by PPAB in vitro.** To identify PPAB that could inhibit the growth of *L. monocytogenes*, we used a modification of the method described by McGarvey et al. (34). Briefly, frozen stock cultures of PPAB in 96-well plates were thawed at 25°C, inoculated into fresh TSB in 96-well plates, and incubated at 37°C for 18 h without shaking. *L. monocytogenes* ATCC 19115 (Thermo Fisher Scientific, Waltham, MA) without heat. A 20-μl aliquot was applied to a 6-mm filter paper
disk and placed onto BHI agar plates previously overlaid with 10 ml of BHI soft agar (0.7% agar) seeded with 0.5 ml of an 18-h culture of each of the L. monocytogenes outbreak strains (RM2387-pNF8, RM2199, RM3180, RM3177, RM3176, RM15994, RM15995, RM15996, RM15997, RM20667, and RM20669) (Table 2), followed by incubation at 30°C for 16 h.

**Growth and persistence of B. amyloliquefaciens ALB65 on cantaloupe melon rinds.** The growth of B. amyloliquefaciens ALB65 on the surfaces of cantaloupe melons was carried out as described previously (34). Briefly, B. amyloliquefaciens ALB65 was grown for 18 h in TSB at 37°C with shaking at 150 rpm and diluted to \(1 \times 10^5\) CFU ml\(^{-1}\) with sterile PBS. Sections (5 by 5 cm) of cantaloupe rinds (~0.5 cm deep) were removed from melons using a sterile scalpel and placed into sterile petri plates (100 × 25 mm deep; Falcon, Corning, NY). Ten l-\(\mu\)l drops of the diluted culture were deposited onto the surface of the melon rinds, followed by incubation at 30°C. The growth of B. amyloliquefaciens ALB65 on the melon rinds was quantified daily for 2 days by homogenizing the sections in 100 ml of PBS in a sterilized blender jar (Oster, Neosho, MO) on low speed for 30 s, followed by 30 s on high speed. The resulting solutions were serially diluted with PBS and plated onto Bacillus cereus selective agar plates (BCA) composed of B. cereus agar base (Oxoid), egg yolk emulsion (Remel), and polymyxin B supplement (Oxoid). The plates were incubated at 37°C for 24 h and counted.

To investigate the ability of B. amyloliquefaciens ALB65 to persist on cantaloupe rinds after a simulated postharvest dunk tank inoculation and subsequent cold storage, we grew B. amyloliquefaciens ALB65 in 600 ml of TSB for 18 h at 37°C with shaking at 150 rpm. The culture, containing approximately \(3 \times 10^6\) CFU ml\(^{-1}\), was placed into a sterile 3.8-liter plastic bag containing a cantaloupe melon to mimic a dump tank washing. The air from the plastic bag was carefully removed by hand so that the culture was in constant contact with the cantaloupe, followed by incubation at 25°C for 30 min (34). After 30 min, the melons were blotted dry with paper towels and air dried 18 h at 25°C. Next, 5-cm\(^2\) sections of the melon rinds were removed using a sterile scalpel and placed into sterile petri plates (100 × 25 mm deep; Falcon), followed by incubation at 8°C. The persistence of B. amyloliquefaciens ALB65 on the melon rinds was quantified every third day for 9 days as described above (34).

To evaluate the persistence of B. amyloliquefaciens ALB65 on the surfaces of preharvest melons, we grew cantaloupe plants (Cucumis melo var. reticulatus) in a greenhouse from seeds (Park Seed, Greenwood, SC) and pollinated them by hand. When the melons were fully formed but still unripe (~30 days postemergence), they were sprayed with 100 ml of an 18-h culture of B. amyloliquefaciens ALB65, containing approximately \(3 \times 10^6\) CFU ml\(^{-1}\), and the number of B. amyloliquefaciens ALB65 CFU on 5-cm\(^2\) sections of the melon rinds was quantified at days 0, 1, 3, and 6 as described above.

**Inhibition of L. monocytogenes growth on cantaloupe melon rinds by B. amyloliquefaciens ALB65.** To evaluate the ability of B. amyloliquefaciens ALB65 to inhibit the growth of L. monocytogenes RM15995, an isolate from the 2011 US multistate cantaloupe outbreak (28), on cantaloupe rinds after a simulated postharvest dunk tank inoculation, we grew B. amyloliquefaciens ALB65 and mimicked a dunk tank inoculation as described above under growth and persistence. PBS was used in place of B. amyloliquefaciens ALB66 cultures for the control melons. Sections (5 cm\(^2\)) of the melon rinds were removed using a sterile scalpel and placed into sterile petri plates (100 × 25 mm deep; Falcon). L. monocytogenes RM15995 was grown for 18 h in BHI at 37°C shaking at 200 rpm and diluted to approximately \(5 \times 10^5\) CFU ml\(^{-1}\) in PBS, and 10 l-\(\mu\)l drops were placed onto the cantaloupe rinds. The rinds were incubated at 30°C for 24 h and quantified for L. monocytogenes by homogenizing the rinds in 100 ml of PBS in a sterilized blender jar at low speed for 30 s, followed by high speed for 30 s using an Osterizer Beehive blender (Oster). The resulting solution was serially diluted in PBS, plated onto modified Oxford agar plus 50 \(\mu\)g/ml nalidixic acid (MOX+Nal) agar plates (Oxoid), incubated at 37°C in a 2.5-liter Oxoid Anaerocar 3.5-liter sacker (Remel) for 2 days, and counted.

To assay for the ability of ALB65 to inhibit the growth of L. monocytogenes strain RM15995 on cantaloupe rinds under simulated postharvest cold storage conditions, we coated cantaloupe melons as described above with B. amyloliquefaciens ALB65 (34), removed 5-cm\(^2\) sections, and inoculated them with L. monocytogenes RM15995 as described above. The rinds were incubated for 6 days at 8°C, and the number of L. monocytogenes RM15995 on the rinds was quantified as described above.

**Identification of B. amyloliquefaciens ALB65 by use of 16S rRNA gene and genomic DNA sequence analysis.** For 16S rRNA gene sequence analysis, B. amyloliquefaciens ALB65 was inoculated into 3 ml of TSB, followed by incubation for 18 h at 37°C shaking at 150 rpm. The culture was centrifuged at 10,000 × g for 10 min, and the pellet was suspended in PBS. DNA was extracted using the Wizard Genomic DNA purification kit (Promega, Madison, WI) according to the manufacturer’s instructions. The 16S rRNA gene was PCR amplified in 50-\(\mu\)l reaction mixtures containing 25 \(\mu\)l of high-fidelity PCR master mix (Roche, Nutley, NJ), 10 ng of DNA, and 10 \(\mu\)M concentrations of the primers 27F (AGAGTTTGATCM TGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) (52) in a C1000/S1000 Touch thermocycler (Bio-Rad, Hercules, CA) under the following conditions: 1 cycle of 95°C for 5 min; 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1.5 min; and 1 cycle of 10 min at 72°C. The resulting DNA was cloned using the TOP10 TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions and transformed into E. coli TOP10 competent cells (Invitrogen). Transformants were grown on LB agar plates containing 50 \(\mu\)g ml\(^{-1}\) kanamycin (Km). Individual colonies were picked and streaked onto LB Km agar plates. Plasmid minipreps were performed using a QIAprep spin miniprep kit according to the manufacturer’s instructions. Plasmid inserts were sequenced using the 27F and 1492R primers and a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). Sequencing reactions were purified using a BigDye XTerminator purification kit (Applied Biosystems); electrophoresis and readout were performed using an Applied Biosystems 3730XL genetic analyzer (Applied Biosystems). The forward
and reverse sequences were aligned using SeqManII software (DNASTAR, Inc., Madison, WI). Sequences were analyzed using the BLAST software and the NCBI nr/nt database set to exclude models and uncultured/environmental sample sequences and to limit to sequences from type material (available at http://blast.ncbi.nlm.nih.gov/).

To resolve B. *amyloliquifaciens* ALB65 to the species level and identify possible antibiotic and secondary metabolite operons, we sequenced the genome using a combination of Pacific Biosciences (PacBio) RS II and MiSeq Illumina platforms (27). Genomic DNA was extracted by the phenol-chloroform method (53). Briefly B. *amyloliquifaciens* ALB65 was cultured for 18 h in 100 ml of TSB at 37°C with shaking at 150 rpm. Cells were harvested by centrifugation at 5,000 × g for 5 min and resuspended in 50 mM Tris buffer (pH 8.0) to an optical density of 600 nm of 1.6 to 1.8. A portion (1.5 ml) of the suspension was transferred to a 15-ml Falcon tube, and 250 μl of 10 mg ml⁻¹ lysozyme solution and 600 μl of 100 mM EDTA (pH 8.0) was added. The mixture was incubated on ice for 10 min; then, 300 μl of 5% SDS was added, and the mixture was vortexed vigorously for 10 s. RNA was removed by the addition of 5 μl of 100 mg ml⁻¹ RNase A, followed by incubation at 37°C for 24 h with intermittent inversion. Next, 10 μl of 15 mg ml⁻¹ proteinase K was added, and the solution was incubated at 37°C for 4 h with inversions every 1 h. Genomic DNA was precipitated with 265 μl of 3.0 M sodium acetate (pH 5.5) and 6 ml of ice-cold ethanol, dried in a Savant SpeedVac concentrator, and resuspended in 400 μl of 10 mM Tris (pH 8.0). DNA was treated with phenol-chloroform-isomyl alcohol (25:24:1), precipitated with 100% ethanol, and resuspended in 100 μl of 10 mM Tris (pH 8.0).

PacBio template preparation was carried out as described for the procedures and checklist for the 20-kb template preparation using the BluePippin size-selection system (53). The genome sequence was assembled and analyzed as described previously (27).

### Data availability
The sequence of the B. *amyloliquifaciens* ALB65 16S rRNA gene was deposited into GenBank (accession number MN538240). The genome sequence of B. *amyloliquifaciens* ALB65 was also deposited in GenBank (accession number CP029069).

#### Statistical analysis.
All experiments were carried out using a complete randomized design. Experimental data were analyzed with one-way analysis of variance analysis using Sigma Plot (SPSS, version 12). The results are presented as mean values ± the standard deviations of at least three independent experiments in which each rind was a replicate.

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