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Rahnella spp. are commonly isolated from Onion (*Allium cepa*) bulbs and are weakly pathogenic

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Abbreviated running title: *Rahnella* spp. in onion (*Allium cepa*)

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Abstract

Aims: Bacterial decays of onion bulbs have serious economic consequences for growers, but the etiologies of these diseases are often unclear. We aimed to determine the role of *Rahnella*, which we commonly isolated from bulbs in the USA and Norway, in onion disease.

Method and Results: Isolated bacteria were identified by sequencing of housekeeping genes and/or fatty acid methyl ester (FAME) analysis. A subset of *Rahnella* spp. strains

were also assessed by multilocus sequence analysis (MLSA); most onion strains belonged to two clades that appear closely related to *R. aquatilis* . All tested strains from both countries caused mild symptoms in onion bulbs but not leaves. PCR primers were designed and tested against strains from known species of *Rahnella.* Amplicons were produced from strains of *R. aquatilis*, *R. victoriana*, *R. variigena*, *R. inusitata*, and *R. bruchi*, and from one of the two strains of *R. woolbedingensis*.

Conclusions: Based on binational testing, strains of *Rahnella* are commonly associated with onions, and they are capable of causing mild symptoms in bulbs.

Significance and Impact of the Study: While *Rahnella* strains are commonly found within field-grown onions and they are able to cause mild symptoms, the economic impact of *Rahnella*-associated symptoms remains unclear.

Keywords: Plant diseases, Plant pathology, PCR (polymerase chain reaction), Pathogenesis, Infection

Introduction

Onions (*Allium cepa*) are susceptible to damage by a number of pests and pathogens, including insects, nematodes, fungi, and bacteria. Decays of onion bulbs caused by bacteria can cause serious economic losses. Bacterial decays can develop in the field during the growing season or in post-harvest storage of the bulbs. Numerous bacteria have been described as onion bulb pathogens, including strains from genera *Burkholderia*, *Enterobacter*, *Pantoea*, *Pseudomonas*, *Pectobacterium*, *Lactobacillus*, and *Leuconostoc* (Schwartz and Mohan, 2007; Bonasera *et al.* 2017).

Onion bulbs with bacterial decay may have any combination of discoloured, water-soaked, macerated, or shrunken scales. In disease caused by macerating bacteria, for example *Burkholderia* spp. or *Dickeya* sp. (Mahenthiralingam *et al.* 2005;

Palacio-Bielsa *et al.* 2007), rotten bulbs can often be identified by visual inspection of intact bulbs or by manually assessing bulb firmness, especially at the bulb neck. In New York State and in Norway, growers often employ skilled workers to hand-sort bulbs and cull any with discernible symptoms of decay.

While macerating bacteria often cause significant damage to bulbs and affect bulb integrity, non-macerating bacteria, for example *Pantoea ananatis* or *Enterobacter* sp. (Carr *et al* 2010; Schroeder and du Toit 2010), may cause internal discolouration of scales. They may slightly reduce the firmness of the bulb neck, but often cause no external symptoms, making them indistinguishable from healthy bulbs during grading. When shipments of bulbs are received by potential buyers, a random sample of bulbs typically is cut and inspected. If inspection reveals unacceptable numbers of symptomatic bulbs, the entire shipment may be rejected. Manual sorting and rejected lots add to the economic impact of bacterial decays of onions on grower profits.

In both New York State and Norway, onion bulbs may be stored for several months after harvest before they are sorted and marketed. In 2010, bacteria were recovered from more than 500 bulbs that had been culled during hand-sorting from cold storage in western New York State. Strains putatively identified as *Rahnella* spp. were recovered from more than 25% of culled bulbs. Also, in Norway, similar surveys yielded *Rahnella* spp. from more than 20% of symptomatic bulbs. In the current work, we determined that strains of *Rahnella spp.* were widely distributed geographically as onion-associated bacteria, and they elicited mild symptoms in artificial inoculation experiments. We isolated several species of *Rahnella* from onions. Most strains clustered into two clades that appear to be closely related to *R. aquatilis*. To facilitate

further work detecting *Rahnella* strains, we developed specific primers and an associated protocol for a polymerase chain reaction (PCR) test.

Materials and methods

Bacterial growth and maintenance (USA)

Bacteria were routinely grown on Luria-Bertani (LB) agar plates and incubated for 1-2 days at 28°C for use in colony PCR or for inoculations of bulbs. For storage of strains, bacteria were transferred from freshly grown plates using sterile cotton-tipped applicators into sterile-filtered 15% glycerol. Bacteria were stored at -80°C.

Bacterial growth and maintenance (Norway)

Bacteria were routinely grown on Nutrient Glucose Agar (NGA) (Lelliott and Stead, 1987). NGA plates were incubated for 1-3 days at room temperature for colony PCR or inoculations of bulbs. For storage of identified strains, bacteria were transferred from freshly grown plates to “protect” vials (Technical Service Consultants, Lancashire, UK) containing ceramic beads. Bacteria were then stored at -80°C.

Isolation of bacteria from onion and environmental samples (USA)

In 2010, growers in western New York State set aside onions with suspected bacterial decay during hand-sorting in cold storage prior to marketing. Approximately 500 bulbs, mostly symptomatic, were sampled at this time. In the winter of 2011-2012, one wooden crate of onions (approximately 400 kg) was selected for sampling from

each of three growers' cold storages in the Elba, NY region. Approximately 100 onions were randomly chosen from those crates three times over the storage season, in October, January, and March. In New York, onions are typically harvested in late August through mid-October. Bulbs were refrigerated until processed by lab personnel. Other samples were occasionally received from onion growers suspecting rot in growing onion plants or recently harvested or stored bulbs. Plants were typically sent to the lab by overnight mail and processed immediately or refrigerated and processed within a few days of arrival.

For onion plants from the field, roots were trimmed, and plants were rinsed with distilled water to remove soil particles. Symptomatic tissues or disease margins were probed with sterile wooden applicators and streaked onto onion extract medium (OEM) (Zaid *et al.* 2012) directly. When dealing with bulbs, they were bisected longitudinally, photographed and assessed for symptoms, and bacterial isolations were made from each bulb. Representatives of the various colony types growing on OEM plates were dilution streaked to purity on LB agar. All incubations were carried out at 28°C.

Strain FC061912-K was isolated from a creek flowing adjacent to an onion field in Western New York. A volume of 400 ml of creek water was centrifuged at 5500 x g for 15 minutes. The resulting pellet was resuspended in 1/100 volume of autoclaved high-purity water, and 100 µl were plated on OEM agar. Colonies of different morphologies were picked and purified by dilution streaking.

Isolation of bacteria from onions (Norway)

The majority of putatively diseased onions were collected from the southeastern part of Norway, in the counties Vestfold, Østfold and Oppland. A smaller number of samples originated from the counties Hedmark, Rogaland and Nord-Trøndelag. Samples were collected from the field during the growing season, directly after harvest, or after storage. In addition, samples were collected from field trials where pathogen control measures with various compounds were being investigated. A total of 368 samples, each consisting of one to 20 onions, typically three to five, were collected during the project period (2012 to 2015), and stored at 5°C until processed.

For onion plants from the field, roots were trimmed, and plants were rinsed with distilled water to remove soil particles. For both growing plants and mature bulbs, symptomatic tissues or the margins between symptomatic and healthy tissue were sampled. Bacteria were released from the sampled tissue by either soaking for 30 minutes in sterile 10 mM phosphate buffered saline, pH 7.2 (PBS) (Anonymous, 2006) or crushing in sterile water. Resulting suspensions were dilution streaked onto NGA.

Onion tissue samples were homogenized in 10-15 ml SPCB buffer (120 mM sodium phosphate, 2 % CTAB, 1.5 M NaCl, pH 8·0) using a Bioreba homogenizer. DNA was isolated from the crude extract using the Kingfisher Duo Prime with KingFisher Cell and Tissue DNA kit, according to the manufacturer's (Thermo Fischer Scientific, Waltham, MA) instructions.

Preliminary Identification of bacteria (USA)

In New York, bulbs harvested from the same field and sampled at the same time were treated as batches. Strains from the same batch of bulbs were grouped based on

similar colony morphologies, digest patterns of amplicons from the DNA gyrase subunit B gene (*gyrB*) as described by Bonasera *et al.* (2014), and by results of indole tests, nitrate reductase and oxidase activities (Schaad *et al.* 2001) and by fluorescence on King's B agar (King *et al.* 1954), modified to contain 0.4 g instead of 1.5 g of $MgSO_4 \cdot 7H_2O$ per liter. Representative strains were chosen from each group, and *gyrB* amplicons obtained by using the 1480F/2242R primer pair (Bonasera *et al.* 2014) were sequenced: amplicons were cleaned using the Clean & Concentrator-5 kit (Zymo Research Corp., Irvine, CA) and sequenced using the *gyrB* 1480F primer, at the Cornell University Biotechnology Resource Center. Resulting sequences were used to search the NCBI Nucleotide collection (nr/nt) database via blastn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Strains with *gyrB* fragment sequences that were most similar to *Rahnella* strains were pursued further.

Preliminary Identification of bacteria (Norway)

Isolates were identified initially by fatty acid methyl ester (FAME) analysis (Sasser, 1990). Of 431 isolates, 130 isolates were also identified by sequencing of a hypervariable region of the 16S ribosomal gene, using primers F985PTO and R1378 and conditions as described previously (Heuer *et al.* 1999, Table 1). Templates were from bacterial colonies supended in 500 µl sterile H_2O and incubated for 10 min at 96ºC. Purified PCR amplicons were sequenced in both directions at GATC Biotech, Germany, using the same primer set as for the PCR amplification. Sequences were assembled, manually edited and aligned using the CLC Main Workbench.

Onion bulb inoculations with *Rahnella* strains

Yellow onion bulbs were purchased from a local grocery store and prepared as described by Schroeder *et al.* (2009). Strains C1b and A66, isolated in North America, were grown on LB agar for 1-2 days at 28°C and swabbed from plates using sterile cotton-tipped applicators into autoclaved high-purity water. Bacterial suspensions were adjusted to OD_{600} of 0.2. Four to five bulbs per strain were injected with 100-500 µl of inoculum using a syringe and 18 gauge needle. Bulbs were incubated at 28-30°C for 10-17 days, after which they were cut longitudinally and assessed for symptoms. Bacteria were recovered from inoculated onion bulbs using LB agar and assessed with Rah 3783 F1/R1 primers or by production of a PCR amplicon using gyrB1480 F/R primers followed by sequencing of the amplicons. Inoculation and re-isolation experiments were completed for strain C1b and A66 three times each, with bacteria recovered from one or two bulbs per assay.

To compare pathogenicity of *Rahnella* strains isolated from Europe and North America, eight isolates of *Rahnella spp.* that had also been included in MLSA (four from Norway and four from the USA) were compared in a pathogenicity test as described above (Figure 2), and scored based on the degree of symptoms (Figure 3). Data were analysed by analysis of variance, and significant differences were separated using Tukeys pairwise comparison (Minitab).

Onion leaf inoculations

Onion plants were grown in an environmental growth chamber as described previously (Bonasera *et al.* 2017). Six leaves of twelve plants were inoculated with

strains C1b or A66 or water by dipping sterile toothpicks in bacterial suspensions or water. These strains were chosen as strains that were isolated early in the study and, based on preliminary analysis of sequencing data from their *gyrB* 1480F/2242R amplicons, both were *Rahnella* and were clearly distinct from each other. Bacterial suspensions were prepared as for onion bulb inoculations. Six plants inoculated with each strain or sterile water were placed in an incubator set to 30°C and six others were placed at room temperature in the laboratory.

Partial *gyrB* sequencing 1480F/2242R

In order to place the Norwegian *Rahnella* strains in context with strains isolated from New York, six strains from Norway were sequenced using the *gyrB* 1480F/2242R primers. Additionally, twelve strains from five different species of *Rahnella* and the closely related bacterium *Ewingella americana* were sequenced with the same primers for use as references. For most strains, these sequences were generated by a single sequencing reaction. Sequences were aligned in Megalign (DNAStar, Madison, WI), and were trimmed to eliminate ambiguous base calls and gaps resulting from poor-quality sequence occurring at the beginning or end of amplicons. Quality of the remaining sequences were then assessed by viewing trace files using FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com). For several strains, additional PCR and sequencing was performed to obtain good-quality sequence over the whole alignment. Sequences generated for this work were deposited in Genbank under accession numbers MK391682-MK391746 and MK408759 (Table S1).

Multilocus sequence analysis (MLSA)

Seven strains from the USA and four strains from Norway that were putatively identified as *Rahnella* spp. were further analysed by MLSA, using partial sequences of four conserved housekeeping genes, *gyrB*, *rpoB* (RNA polymerase β subunit), *infB* (translation initiation factor IF-2), and *atpD* (ATP synthase subunit beta). In an effort to place *Rahnella* onion isolates in context with existing sequence data of *Rahnella* spp., amplicons with coverage that included the sequence positions used in the MLSA published by Brady *et al.* (2014) were obtained. Consequently, a combination of previously published and new primers were used (Table 1), as not all primer pairs from Brady *et al.* (2014) worked well with the conditions used in this study. PCRs to generate amplicons for sequencing were generally performed in 24 µl volumes, using 12.18 µl of water, 4.8 µl 5x OneTaq GC buffer (New England Biolabs, Ipswich, MA), 2.4 µl 2.5 mM dNTPs, 1.25 µl each of the forward and reverse primers, 0.12 µl of OneTaq (New England Biolabs), and 2 µl template. For each novel sequence used in the MLSA (GenBank accession nos. MK387392-MK387415, Table S2), two amplicons produced in separate reactions were sequenced as described above. Contigs were assembled using SeqMan Pro version 12.2.0 or 13.0.0 (DNAStar). Groups of sequences were aligned in MegAlign or using the “align two or more sequences” option for the blastn tool (https://blast.ncbi.nlm.nih.gov/). Sequences were trimmed to match the coverage of previously reported *Rahnella* MLSA sequences (Brady *et al.* 2014) and concatenated in the following order: *gyrB*, *rpoB*, *infB*, and *atpD*. The *gyrB* sequences used in MLSA are upstream of and do not overlap with the sequences obtained from the *gyrB* 1480F/2242R amplicons.

236

237 **Generation of phylogenetic trees**

238 Separate phylogenetic trees were constructed for the *gyrB* 1480F/2242R
239 amplicon sequences (Table S1, Figure S1) and for the concatenated MLSA sequences
240 (Table S2, Figure 1). Sequences were aligned using the ClustalW method according to
241 default parameters, and phylogenetic trees were generated using MEGA version 7.0.26
242 (Kumar *et al.* 2016). There were no gaps in the alignments. The evolutionary history
243 was inferred using the Maximum Likelihood method based on the Tamura-Nei model
244 (Tamura and Nei, 1993). Initial tree(s) for the heuristic search were obtained
245 automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise
246 distances estimated using the Maximum Composite Likelihood (MCL) approach, and
247 then selecting the topology with superior log likelihood value.

248

249 **Design of *Rahnella*-specific primers**

250 The genomes of *Rahnella aquatilis* CIP 78.65 = ATCC 33071 (GenBank
251 accession no. CP003244.1) (Martinez *et al.* 2012a) and *Serratia proteamaculans* 568
252 (CP000826.1) were aligned using Progressive Mauve, Mauve version 2.3.1 build 173
253 (Darling *et al.* 2010). Strains of *Serratia* are relatively close relatives to *Rahnella*, and
254 are occasionally isolated from onions. The *Serratia* strain was included in the
255 comparison in order to exclude genes that are conserved outside of the genus
256 *Rahnella.* Genes annotated as “hypothetical proteins” and present in the *Rahnella* strain
257 but not in the *Serratia* strain were used to search the NCBI Genomes database using
258 blastn. Genes present in the three *Rahnella* genomes available at the time, *R. aquatilis*

ATCC 33071, *Rahnella* sp. Y9602, and *R. aquatilis* HX2, but not in other available genomes were used to search the NCBI Whole Genome Shotgun (WGS) database and filtered based on length (at least 300 bp). Putative genes that appeared to be unique to the three sequenced *Rahnella* strains were considered good target regions for designing specific primers. Similar sequences from strains ATCC 33071, Y9602, and HX2 were aligned using MegAlign, and well-conserved portions of three genes, Rahaq2_0130, Rahaq2_3783, and Rahaq2_3707, were selected for primer design. Target regions were manually chosen, and annealing temperature and predicted annealing sites within the target genes were assessed using PrimerSelect (DNAStar). Potential primers were checked for specificity to *Rahnella* by searching specifically genomes from the Enterobacteriaceae (taxid:543), Pseudomonadales (taxid:72274), and Burkholderiaceae (taxid:119060) using the Primer-BLAST tool from NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi). Primer pair Rah 3783 F1/R1 (Table 1), designed to amplify part of the gene designated Rahaq2_3783 in strain ATCC 33071, yielded single amplicons of the expected size, 525 base pairs (bp), from six *Rahnella* strains in preliminary experiments. This pair was further assessed for specificity and sensitivity.

Assessment of *Rahnella*-specific primers

Bacterial suspensions for use in colony PCR were prepared by touching sterile wooden applicators five times to ribbons of bacterial growth on LB agar plates and swirling the applicator into 200 µl of sterile high-purity water. The resulting suspensions were slightly cloudy and used as templates in PCR directly.

Each 12 µl reaction contained 5.09 µl water, 2.4 µl 5x OneTaq GC buffer (New England Biolabs, Ipswich, MA), 1.2 µl 2.5 mM dNTPs, 0.625 µl 10 µM Rah 3783 F1 primer, 0.625 µl 10 µM Rah 3783 R1 primer, 0.06 µl OneTaq DNA Polymerase (New England Biolabs), and 2 µl of template. Amplification was performed with one cycle at 95°C for 10 min; 45 cycles of: 95°C for 30 s, 57°C for 45 s, 72°C for 50 s; and a cycle of 72°C for 10 min. PCR products were analysed following electrophoresis through a 1% agarose gel.

To assess specificity of the Rah3783 F1/R1 primer pair, 19 strains of *Rahnella* spp. isolated from onions, selected from different branches of a phylogenetic tree based on partial *gyrB* sequence, were used for testing. In addition, 11 strains from 8 other genera documented as onion pathogens (*Xanthomonas axonopodis* pv. *allii*, *Pseudomonas viridiflava*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pantoea ananatis*, two strains of *Pantoea agglomerans*, *Erwinia rhapontici*, *Enterobacter* sp., *Dickeya dadantii*, *Burkholderia gladioli* pv. *alliicola*, and *Burkholderia cepacia*) were included in the primer testing, as well as 10 reference strains of *Rahnella* spp. and two of *E. americana*. This experiment was repeated three times.

Primer sensitivity of the Rah3783 F1/R1 primer pair was determined against bacterial suspensions of *Rahnella* sp. Y9602 in sterile water. Bacterial suspensions were adjusted to an optical density at 600 nm (OD_{600}) of 0.2 (approximately 10^8 CFU/ml) and were serially diluted in 10-fold steps ranging approximately 10^8 to 10^1 CFU/ml. Volumes of 5 µl from each dilution were spotted five times each onto LB agar and incubated overnight at 28°C to obtain colony counts of viable bacteria. PCRs of 12 µl were prepared as stated above. Undiluted, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} dilutions of

bacterial suspension and water were used as templates. This experiment was repeated three times.

Results

Recovery of *Rahnella spp.* from naturally infected onion bulbs and plants

Rahnella strains were frequently recovered from onions in both the USA and Norway. In western NY in 2010, *Rahnella* strains were recovered from 136 of 508 culled bulbs assessed (27%). This initial survey prompted an additional study of bacteria from onion bulbs. In the winter of 2011-2012, onion bulbs were randomly sampled from growers' overwinter storage in western NY. Bulbs were sampled early (late October), midway (late January), and late (mid-March) in the storage season. *Rahnella* strains were recovered from both healthy and symptomatic bulbs at low levels. Bulbs from which *Rahnella* strains were isolated ranged from nonsymptomatic to completely discoloured with severe maceration (Figure S2). *Rahnella* strains were recovered from 9 of 748 (1%) of healthy-appearing and 25 of 150 (17%) of symptomatic bulbs (Table S3). *Rahnella* strains were often isolated together with other genera of bacteria. In Norway *Hafnia* sp. and *Serratia* sp. were most often isolated together with *Rahnella*, while in the USA *Pseudomonas* spp. were most often isolated together with *Rahnella* strains.

In a 4-year Norwegian survey of 368 samples of groups of one to twenty symptomatic bulbs, 109 isolates were identified as *R. aquatilis* by FAA and/or 16S sequencing. The FAA similarity index was > 0.8 for *R. aquatilis*. These were confirmed with 16S rRNA sequences, which were 100% similar to a number of different *R. aquatilis* isolates.

Pathogenicity of Rahnella strains

Attempts to infect onion leaves using *Rahnella* strains C1b and A66 were not successful. Currently, there is no evidence that *Rahnella* strains are capable of causing leaf lesions (data not shown).

Artificially inoculated yellow onion bulbs showed symptoms ranging from mild discolouration along the inoculation site to water-soaking and discolouration of one or a few internal scales, but the bulbs generally remained firm and without signs of maceration. Symptoms were distinct from sterile water-injected negative controls. Severity of symptoms were not completely consistent, sometimes resulting in more severe symptoms (Figure S3). Bacteria recovered produced an amplicon of appropriate size with *Rahnella*-specific primers or produced *gyrB* 1480F/2242R amplicons with identical sequences to those of the inoculated strains.

Additional inoculations were performed to compare pathogenicity of *Rahnella* strains recovered from the USA and Norway. The results showed water-soaking and discolouration (from light to dark brown); in some cases, scale shrinkage was observed. In a side-by-side comparison of strains from USA and Norway, there were no significant differences in virulence (Figure 3).

Phylogenetic analysis

In the routine course of identifying bacteria from onions, we generated sequence for the *gyrB* 1480F/2242R amplicon from numerous strains of *Rahnella.* To assess the utility of these sequences in identifying strains of *Rahnella* to species level, partial *gyrB*

sequences generated for verified strains of *R. aquatilis*, *R. victoriana*, *R. variigena*, *R. inusitata*, *R. bruchi*, *R. woolbedingensis*, and *E. americana*, or downloaded from GenBank. Phylogenetic trees were generated and showed that most isolates from onions (originating from both North America and Europe) formed a group containing three major clades. Six strains clustered tightly with *R. aquatilis*, 17 strains formed a separate clade with *R. aquatilis* as its nearest neighbor, and 27 strains clustered with *Rahnella* sp. Y9602 (Figure S1).

MLSA was performed on a subset of *Rahnella* strains isolated from the USA and Norway to conclusively identify them. Strains were placed into context with different *Rahnella* species based on sequence data available in GenBank (Brady *et al.* 2014), using the MLSA scheme designed by Brady *et al.* (2008). Of the ten *Rahnella* strains isolated from onions and used in MLSA, one strain (AR25a) clustered tightly with *R. aquatilis*, three additional strains (L57-1-12, SL6, and A66) formed a separate clade near *R. aquatilis*, four strains (L31-1-12, L172-1A, C1b, F57b) clustered with *Rahnella* sp. Y9602, one strain (G37d) clustered with *R. victoriana* strains, and one (H11b) did not cluster well with any of the reference strains. An additional strain (FC61912-K) was isolated from a creek flowing adjacent to an onion field; it clustered loosely with *R. inusitata* (Figure 1). Strains from onion that were represented in both the MLSA and *gyrB* tree grouped to the same previously-characterized *Rahnella* strains in both trees.

Rahnella-specific primers

The Rah3783 primer pair produced amplicons of approximately 500 bp (expected size 525 bp) from 22 of 23 *Rahnella* strains isolated from onions, including all strains

from the clades containing most onion isolates. Among these 23 strains, six were from Norway, fourteen from New York, and three from Oregon. The strain (H11b) that did not produce an amplicon with the Rah3783 primer pair did not cluster with the majority of onion isolates and did not cluster tightly with any reference strains of *Rahnella*. Additionally, reference strains from *R. aquatilis*, *R. victoriana*, *R.variigena*, *R. inusitata*, *R. bruchi*, *R. woolbedingensis*, and *E. americana* were tested. All *Rahnella* strains produced a fragment of the expected size except one of the two strains of *R. woolbedingensis.* The *E. americana* strains did not produce a fragment (Figure 4, Table 2).

None of the 11 strains from other bacterial genera documented as onion pathogens (*X. axonopodis* pv. *allii*, *P. viridiflava*, *P. carotovorum* subsp. *carotovorum*, *P. ananatis*, *P. agglomerans*, *E. rhapontici*, *Enterobacter* sp., *D. dadantii*, *B. gladioli* pv. *alliicola*, and *B. cepacia*) produced amplicons (Table 2).

The minimum amount of *Rahnella* sp. strain Y9602 that could be reliably amplified using the Rah3783 primer pair was an average of 7,600 CFU/reaction. A ten-fold dilution of that template yielded no band or only faintly discernible bands.

The PCR assay was also tested on onion samples from Norway that had varying degrees of symptoms. Of 88 samples tested, 64 were positive, 5 were weakly positive and 19 were negative for *Rahnella* spp. Samples with no symptoms were used as controls and did not give any PCR product with the *Rahnella*-specific primers. The assay successfully detected *Rahnella* sp. in onion samples, and hence may prove to be a valuable tool for identification, detection and epidemiological studies of the bacterium.

Discussion

"Bacterial decay" in onions is an umbrella term describing onion bulb disease symptoms consistent with bacterial infection, in the absence of detectable fungal or insect problems. The symptoms caused by the various known bacterial decay pathogens are not easily distinguishable, with many pathogens causing water-soaking and discolouration of bulb scales and several causing maceration (Schwartz and Mohan, 2007). Similar conditions are favorable for multiple bacterial decay pathogens, such as wounded leaves, high relative humidity, free water, and high temperatures (Schwartz and Mohan, 2007). Loss of plant tissue integrity associated with infection can also make onions more vulnerable to additional colonization by secondary invaders (Brewster, 2008). Finally, endophytic bacteria that may exist in relatively low numbers in otherwise healthy onion bulbs may grow more rapidly in stressed or compromised tissue, resulting in opportunistic infection (Cother and Dowling, 1986). Examples of opportunistic bulb diseases are known: Enterobacter bulb decay and internal brown rot of onions (caused by *Pseudomonas aeruginosa*) have been described as opportunistic infections or as only occurring under special conditions. (Bishop and Davis 1990; Cother *et al*. 1976)

Growers describe bacterial decays in growing onions and harvested bulbs as a problem that has caused increasing losses in the last 15-20 years. The reasons behind the increased losses are unknown but may involve a combination of factors, including emergence of new pathogens, changing cultural practices, the introduction of new onion cultivars, and changing climate. Because of the increased problems with bacterial decays and because of the possibility of identifying emerging pathogens in onion-

growing regions, researchers in the USA and Norway separately investigated which bacteria were commonly associated with diseased onion bulbs in their regions and whether these commonly-detected bacteria represented substantial threats to onion production.

Rahnella strains were some of the most commonly isolated bacteria from diseased onion bulbs in both the USA and Norway. A subsequent survey of randomly chosen onion bulbs from growers' storage revealed that *Rahnella* strains could be isolated from both symptomatic and healthy-appearing bulbs. Recently, researchers in Nova Scotia, Canada also detected *Rahnella* strains from both healthy and symptomatic bulbs from growers' storage (Yurgel *et al.* 2018). In our study, the frequency with which *Rahnella* strains were isolated from symptomatic bulbs was 17% versus only 1% for healthy bulbs. The relatively greater abundance of *Rahnella* strains suggests a relationship between the growth of *Rahnella* in onion bulbs and the presence of disease symptoms. However, it was unclear whether *Rahnella* strains were involved in the disease process directly or whether *Rahnella* strains are particularly capable of colonizing or multiplying within diseased onion bulbs.

In this study, *Rahnella* strains were isolated from onion bulbs exhibiting a range of symptoms, from mild discolouration of one or a few scales to water soaking and maceration of entire bulbs. However, in laboratory inoculations of healthy-appearing bulbs, pure cultures of *Rahnella* strains typically caused mild symptoms, indicating that additional bacteria or fungi were probably responsible for the most severe symptoms in bulbs from which *Rahnella* strains were isolated. *Rahnella* strains may therefore exist as endophytes that are opportunistically pathogenic to onion bulbs, and their ability to

443 cause mild symptoms, including scale discolouration and shrinkage, may predispose
444 bulbs to disease caused by other pathogens. Alternatively, *Rahnella* strains may be
445 particularly adept at colonizing bulbs with symptoms caused by other pathogens. More
446 work is needed to tease apart these possibilities, which are not mutually exclusive.
447 A number of factors may influence the composition of the viable microbes in an
448 onion bulb, including susceptibility of the host to infection, inter-species competition,
449 antibiosis, and external environmental factors. Onion storage facilities are designed to
450 keep bulbs at low temperature, either by refrigeration, or by use of louvers that allow
451 cold winter air into the storage facility. Onion bulb storage at low temperature may be
452 particularly favorable for *Rahnella* strains compared to other bacteria. Strains of
453 *Rahnella* are considered psychrotrophic and have previously been described as
454 spoilage bacteria for foods stored under refrigerated conditions of 4-5°C (Jensen *et al.*
455 2001; Ercolini *et al.* 2006). During cold growing seasons or in growers' storage during
456 the winter months, *Rahnella* strains might be expected to survive or multiply better than
457 other bacteria, including virulent onion pathogens. The conditions under which onion
458 bulbs were stored may have contributed to the frequent isolation of *Rahnella* strains
459 from bulbs in this study.
460 In addition to being tolerant of a wide range of growth temperatures, strains of
461 the genus *Rahnella* are able to occupy many niches successfully. *Rahnella* spp. strains
462 have been isolated from many different substrates, including soil, water, insects, plants,
463 and people (Brady *et al.* 2014). Some species of *Rahnella* have also previously been
464 described as commonly associated with diseased plant tissue. *R. victoriana* in particular
465 is commonly associated with trees suffering from acute oak decline in the UK, but the

disease appears to be caused by a complex of species and the particular role of *R. victoriana* in the disease is not clear (Denman *et al.* 2017). This acute oak decline situation bears some resemblance to observations in this study, in which strains of *Rahnella* were more commonly isolated as a component of the bulb microbiome from diseased, rather than healthy plant tissue, yet *Rahnella* strains produced only mild symptoms in pathogenicity tests in the laboratory. Indeed, the difficulty in isolating known virulent pathogens from many symptomatic bulbs, and the existence of onion diseases that become problematic under particular storage conditions and are caused by bacteria that can frequently be isolated from healthy bulbs (for example, Enterobacter bulb decay), suggests that bulb decays may sometimes be caused by complexes of opportunistically pathogenic endophytic bacteria.

While multiple species of *Rahnella* were isolated from onions in the course of these studies, the majority of strains belonged to a monophyletic group consisting of three clades represented by the type strain of *R. aquatilis*, the genome-sequenced strain *Rahnella* sp. Y9602, and a branch that may represent an undescribed species cluster. This group of strains from onions collected in different years and from across vast geographic distances suggests that these strains share features that allow successful colonization and survival within onions that strains outside of this group lack. This work suggests that this particular group of *Rahnella* strains, specifically *R. aquatilis* and two closely-related species, have diseased onion bulbs as a niche. In the future, comparison of genomes from onion-associated *Rahnella* strains might suggest suites of genes involved in successful colonization of onion tissues. Primers developed in this

work should help to advance future studies by aiding in the rapid screening for onion-associated *Rahnella* strains.

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Conflict of Interest

No conflict of interest declared.

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599
600 **Table 1** Primers used in this study

	Sequence	Annealing temperature used	Amplicon size	Reference
Primers for multilocus sequence analysis (MLSA)				
gyrB 01-Fs	TAA RTT YGA YGA YAA CTC YTA YAA AGT	45°C	971 bp	Brady *et al*. (2008)
gyrB 02-R	CMC CYT CCA CCA RGT AMA GTT			Brady *et al*. (2008)
infB 01-F	ATY ATG GGH CAY GTH GAY CA	48°C	1124 bp	Brady *et al*. (2008)
infB 02-R	ACK GAG TAR TAA CGC AGA TCC A			Brady *et al*. (2008)
rpoB 2522-2543 R	TCA GGC CCT AAC TTG GTG TCA C	53°C	1514 bp	this study
rpoB 1030-1049 F	GGC GCG TAC ATG TCC GAG AC			this study
atpD 922-943 R	GAG CGA AGG TGG TAG CTG GAG A	53°C	907 bp	this study
atpD 37-57 F	GTG GTG GAC GTC GAG TTC CCT			this study
atpD 58-81 F	CAG GAT GCA GTA CCG AAC GTG TAC	N/A	N/A	this study
atpD 900-921 R	TGG GTC AGT CAA GTC ATC CGC A	N/A	N/A	this study
rpoB 1307-1325 F	GTA ACG GCC AGG GCG AAG T	N/A	N/A	this study
rpoB 2138-2159 R	CGT TTG GCT ACG GCA GTC ACA C	N/A	N/A	this study
infB 1236-1257 F	CTC ATT GCT TGA CTA CAT TCG T	N/A	N/A	this study
infB 2092-2116 R	CCT GAA CGT CTG ACT TCA GAA CAA T	N/A	N/A	this study
Primers to amplify *gyrB* fragments for preliminary identification of strains				
gyrB 1480F	GGC ATC ATC ATC ATG ACC GA	50°C	788 bp	Bonasera *et al*. (2014)
gyrB 2242R	GTS GTT TCC CAS AGC TG			Bonasera *et al*. (2014)
Rahnella-specific primers				
Rah 3783 F1	CGG GAT CGT CCG TTA TAA AGG CA	57°C	524 bp	this study
Rah 3783 R1	ACG GTG CGT CCG TTC AGA TCA CC			this study
16S rDNA primers				
F985PTO	AAC GCG AAG AAC CTT AC	55°C	434 bp	Heuer *et al*. (1999) - modified
R1378	CGG TGT GTA CAA GGC CCG GGA ACG			Heuer *et al*. (1999)

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603 **Table 2** Detection of strains with *Rahnella*-specific primers

Species	Strain	Isolated from	Received from / Reference	Rah 3783 F1/R1 amplicon
Rahnella strains isolated from onions				
Rahnella sp.	A66	culled onion from storage NY, USA	this study	+
Rahnella sp.	A78	culled onion from storage NY, USA	this study	+
Rahnella sp.	AG1a	isolated from onion tplant, NY USA	this study	+
Rahnella sp.	AP10b	isolated from onion plant NY, USA	this study	+
Rahnella sp.	AR16b	isolated from onion bulb grown in OR, USA	this study	+
Rahnella sp.	AR20	isolated from onion bulb grown in OR, USA	this study	+
Rahnella sp.	AR25a	isolated from onion bulb grown in OR, USA	this study	+
Rahnella sp.	C10	culled onion from storage NY, USA	this study	+
Rahnella sp.	C1b	culled onion from storage NY, USA	this study	+
Rahnella sp.	E32Ma	culled onion from storage NY, USA	this study	+
Rahnella sp.	F57b	culled onion from storage NY, USA	this study	+
Rahnella sp.	G37d	culled onion from storage NY, USA	this study	+
Rahnella sp.	G4	culled onion from storage NY, USA	this study	+
Rahnella sp.	G42	culled onion from storage NY, USA	this study	+
Rahnella sp.	H11b	culled onion from storage NY, USA	this study	-
Rahnella sp.	H23	culled onion from storage NY, USA	this study	+
Rahnella sp.	I50b	freshly harvested onion bulb NY, USA	this study	+
Rahnella sp.	L151-1a	onion from county of Østfold, Norway	this study	+
Rahnella sp.	L172-1A	onion from county of Vestfold, Norway	this study	+
Rahnella sp.	L173-1B	onion from county of Vestfold, Norway	this study	+
Rahnella sp.	L31-1-12	onion from county of Vestfold, Norway	this study	+
Rahnella sp.	L57-1-12	onion from county of Oppland, Norway	this study	+
Rahnella sp.	SL6	onion from county of Hedmark, Norway	this study	+
Rahnella strains isolated from other sources				
Rahnella sp.	FC61912-K	Creek water, NY, USA	this study	+
R. victoriana	FRB 225^T	*Quercus robur*, symptomatic inner bark, Suffolk, UK	Brady *et al*. (2014)	+
R. victoriana	USA 13	*Quercus kelloggii*, symptomatic inner bark, California, USA	Brady *et al*. (2014)	+
R. variigena	FOD 20/8	*Quercus robur*, wound response fluid, Gloucestershire, UK	Brady *et al*. (2014)	+
R. variigena	PFK 1/1C2a	*Quercus robur*, symptomatic inner bark, Sussex, UK	Brady *et al*. (2014)	+
R. inusitata	FOD 9/5a	*Quercus robur*, symptomatic inner bark, Gloucestershire, UK	Brady *et al*. (2014)	+
R. inusitata	FOD 9/21	*Quercus robur*, symptomatic inner bark, Gloucestershire, UK	Brady *et al*. (2014)	+
R. bruchi	FRB 226^T	*Agrilus biguttatus*, gut, Shropshire, UK	Brady *et al*. (2014)	+

R. bruchi	ALN 45	*Alnus glutinosa*, inner bark, Surrey, UK	Brady *et al*. (2014)	+
R. woolbedingensis	FRB 227^{T}	*Alnus glutinosa,* inner bark, Surrey, UK	Brady *et al*. (2014)	+
R. woolbedingensis	WAL 10	*Juglans regia*, inner bark, Surrey, UK	Brady *et al*. (2014)	-
Other bacteria				
Ewingella americana	FOD 24/3b	*Quercus robur,* symptomatic inner bark, Gloucestershire, UK	Brady *et al*. (2014)	-
Ewingella americana	AT 14b	*Quercus robur*, symptomatic inner bark, Shropshire, UK	Brady *et al*. (2014)	-
Burkholderia cepacia	ATCC 25416	Onion, 1948	type strain of *B. cepacia*	-
Burkholderia gladioli pv. alliicola	ATCC 19302	Onion bulb rot, USA	type strain of *B. gladioli*	-
Dickeya dadantii	Dickey 151			-
Enterobacter sp.	EcWSU1	Onion, USA	Humann *et al*. (2011)	-
Erwinia rhapontici	ATCC 29283	Rhubarb, England	type strain of *E. rhapontici*	-
Pantoea agglomerans	SUH1	Onion, South Africa	Hattingh and Walters (1981)	-
Pantoea agglomerans	ATCC 27155	Knee laceration	type strain of *P. agglomerans*	-
Pantoea ananatis	ATCC 33244; LMG 2665	Pineapple, Brazil	type strain of *P. ananatis*	-
Pectobacterium carotovorum	ATCC 15713	Potato, Denmark	type strain of *P. carotovorum*	-
Pseudomonas viridiflava	LMG 2352	Dwarf or runner bean, Switzerland	type strain of *P. viridiflava*	-
Rahnella aquatilis	ATCC 33071	Drinking water, France	type strain of *R. aquatilis*	-
Xanthomonas axonopodis pv. *allii*	O274	Onion, CO, USA	H. Schwartz, Colorado State University	-

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607 **Figure 1** Multilocus sequence analysis tree of 11 *Rahnella* strains isolated from onion or
608 a creek running adjacent to an onion field shown in context with published *Rahnella*
609 strains. Strains isolated from onions are highlighted in yellow. The tree with the highest
610 log likelihood (-11128.21) is shown. The percentage of trees in which the associated
611 taxa clustered together is shown next to the branches. The tree is drawn to scale, with
612 branch lengths measured in the number of substitutions per site. The analysis involved
613 27 strains. There were a total of 2635 positions in the final dataset. *Xenorhabdus*
614 *nematophila* ATCC is used as an outgroup. Analysis was performed with concatenated
615 sequences from *gyrB* (gyrase subunit B gene), *rpoB* (RNA polymerase β subunit), *infB*
616 (translation initiation factor IF-2), and *atpD* (ATP synthase subunit beta) genes. There
617 were a total of 2636 positions in the dataset. N: Isolated from onions in Norway; OR:
618 Isolated from onions grown in OR, USA; NY: Isolated from onions grown in NY, USA;
619 W: Isolated from creek water adjacent to onion field, NY, USA.

620 **Figure 2** Five onion bulbs each were inoculated with 8 *Rahnella* strains recovered from
621 Norway and the USA. Symptoms were generally mild. Strains from Norway (L31-1-12,
622 L57-1-12, SL6, and L172-1a,) and USA (A66, AR25a, F57b, and C1b).

623 **Figure 3** Symptoms in bulbs from experiments with strains from the USA and Norway.
624 A. Three scoring categories were established (from left to right): 0 = no symptoms; 1 =
625 weak discolouration; 2 = darker discolouration and scale shrinkage. B. Results from
626 pathogenicity test of isolates from the USA and Norway.

627 **Figure 4** Example of agarose gel with PCR products amplified using *Rahnella* specific
628 primers Rah 3783 F1/R1. L: 2-log ladder (New England Biolabs), 1: *R. victoriana* FRB

225T, 2: *R. victoriana* USA 13, 3: *R. variigena* FOD 20/8, 4: *R. variigena* PFK 1/1C2a, 5: *R. inusitata* FOD 9/5a, 6: *R. inusitata* FOD 9/21, 7: *R. bruchi* FRB 226T, 8: *R. bruchi* ALN 45, 9: *R. woolbedingensis* FRB 227T, 10: *R. woolbedingensis* WAL 10, 11: *E. americana* FOD 24/3b, 12: *E. americana* AT 14b, 13: *Rahnella* sp. C1b, 14: water control.

Supporting Information

Table S1 Accession numbers for partial gyrase B sequence derived using the gyrB 1480F/2242R primers

Table S2 Accession numbers for sequences used in multilocus sequence analysis

Table S3 Numbers of bulbs from which particular genera of bacteria were recovered from surveys of diseased and healthy onion bulbs in USA

Figure S1 Samples of bulbs from which *Rahnella* strains were recovered in screen of random bulbs from growers' cold storage in NY. A. Examples of bulbs from which only *Rahnella* strains were recovered. B. Examples of bulbs from which both *Rahnella* strains and other bacteria were recovered.

Figure S2 Maximum Likelihood tree using partial *gyrB* sequence. Strains isolated from onion are highlighted in yellow. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, and the units of branch lengths are the number of substitutions per site. The analysis involved nucleotide sequences from 71 strains. There were a total of 625 positions in the final dataset. N: Isolated from onions in Norway; OR: Isolated from onions grown in OR,

651 USA; NY: Isolated from onions grown in NY, USA; W: Isolated from creek water
652 adjacent to onion field, NY, USA.
653 **Figure S3** Bulbs syringe-inoculated with water (A), *Rahnella* sp. C1b (B), and
654 *Enterobacter* sp. EcWSU1 (C). Symptoms elicited by *Rahnella* are typically mild. The
655 bulbs presented in (B) had severe symptoms compared to other repetitions of the assay
656 (see Figure 2). The reasons for between-assay variations in severity are unknown but
657 could be due to variations in host susceptibility due to bulb age or genotype. Variation
658 in the symptom severity between assays adds to the difficulties in assessing the real-
659 world impacts of *Rahnella* spp. bacteria on onion production. Strains inoculated with
660 *Enterobacter* sp. EcWSU1 are included for comparison with a known opportunistic
661 pathogen of onion bulb.
662

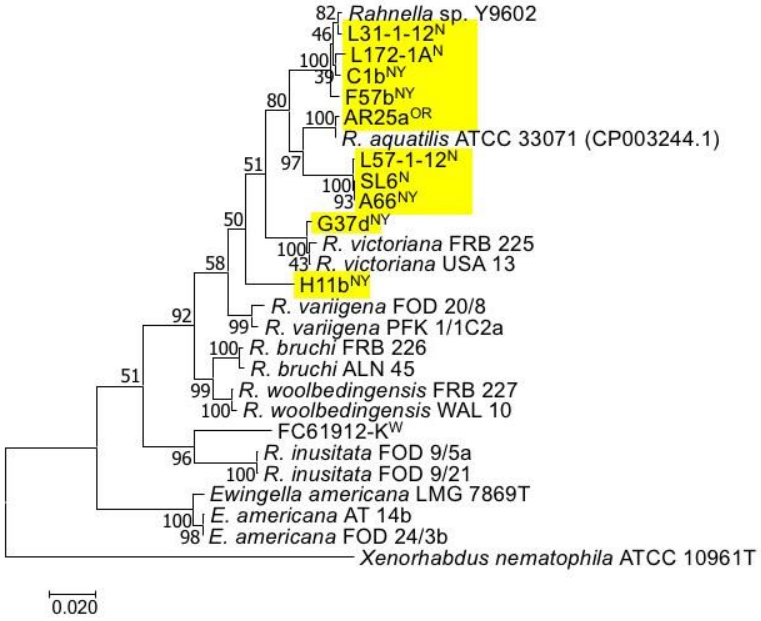

Figure 1 Multilocus sequence analysis tree of 11 *Rahnella* strains isolated from onion or a creek running adjacent to an onion field shown in context with published *Rahnella* strains. Strains isolated from onions are highlighted in yellow. The tree with the highest log likelihood (-11128.21) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 27 strains. There were a total of 2635 positions in the final dataset. *Xenorhabdus nematophila* ATCC is used as an outgroup. Analysis was performed with concatenated sequences from *gyrB* (gyrase subunit B gene), *rpoB* (RNA polymerase β subunit), *infB* (translation initiation factor IF-2), and <>atpD (ATP synthase subunit beta) genes. There were a total of 2636 positions in the dataset. N: Isolated from onions in Norway; OR: Isolated from onions grown in OR, USA; NY: Isolated from onions grown in NY, USA; W: Isolated from creek water adjacent to onion field, NY, USA.

190x254mm (96 x 96 DPI)

Figure 2 Five onion bulbs each were inoculated with 8 *Rahnella* strains recovered from Norway and the USA. Symptoms were generally mild. Strains from Norway (L31-1-12, L57-1-12, SL6, and L172-1a,) and USA (A66, AR25a, F57b, and C1b).

190x254mm (96 x 96 DPI)

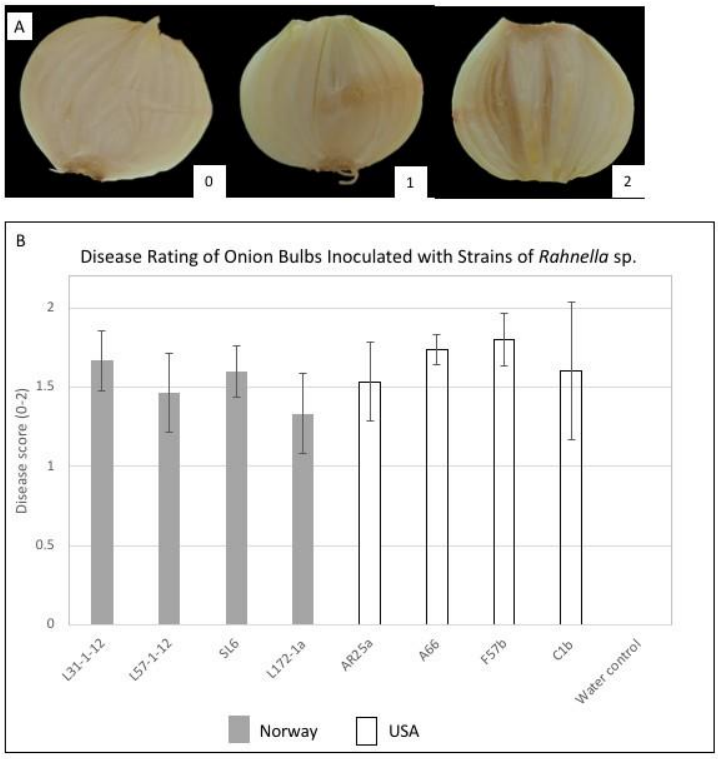

Figure 3 Symptoms in bulbs from experiments with strains from the USA and Norway. A. Three scoring categories were established (from left to right): 0 = no symptoms; 1 = weak discolouration; 2 = darker discolouration and scale shrinkage. B. Results from pathogenicity test of isolates from the USA and Norway.

190x254mm (96 x 96 DPI)

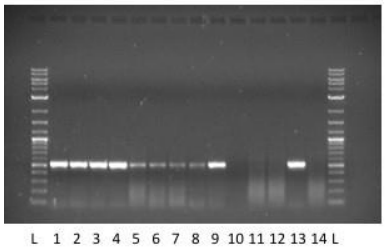

Figure 4 Example of agarose gel with PCR products amplified using *Rahnella* specific primers Rah 3783 F1/R1. L: 2-log ladder (New England Biolabs), 1: *R. victoriana* FRB 225T, 2: *R. victoriana* USA 13, 3: *R. variigena* FOD 20/8, 4: *R. variigena* PFK 1/1C2a, 5: *R. inusitata* FOD 9/5a, 6: *R. inusitata* FOD 9/21, 7: *R. bruchi* FRB 226T, 8: *R. bruchi* ALN 45, 9: *R. woolbedingensis* FRB 227T, 10: *R. woolbedingensis* WAL 10, 11: *E. americana* FOD 24/3b, 12: *E. americana* AT 14b, 13: *Rahnella* sp. C1b, 14: water control.

190x254mm (96 x 96 DPI)

Table S1 Accession numbers for partial gyrase B sequence derived using the gyrB 1480F/2242R primers

Species	Strain	Isolated from	Received from / Reference	Accession
Rahnella sp.	Y9602	soil at U.S. Dept. of Energy Oak Ridge Reservation in Oak Ridge, TN	Martinez *et al.* (2007)	CP002505.1
Rahnella sp.	Q73b	symptomatic red onion bulb from cold storage NY, USA	this study	MK391682
Rahnella sp.	R27c	symptomatic yellow onion bulb recovered from storage NY, USA	this study	MK391683
Rahnella sp.	R92a	symptomatic yellow onion bulb from storage NY, USA	this study	MK391684
Rahnella sp.	T11a	symptomatic yellow onion bulb from storage NY, USA	this study	MK391685
Rahnella sp.	T100a	symptomatic yellow onion bulb from storage NY, USA	this study	MK391686
Rahnella sp.	A12a	culled red onion from storage NY, USA	this study	MK391687
Rahnella sp.	A66	culled onion from storage NY, USA	this study	MK391688
Rahnella sp.	A78	culled onion from storage NY, USA	this study	MK391689
Rahnella sp.	AG6b	symptomatic bulb tissue from growing onion NY, USA	this study	MK391690
Rahnella sp.	AR20	onion bulb grown in OR, USA	this study	MK391691
Rahnella sp.	AR25a	onion bulb grown in OR, USA	this study	MK391692
Rahnella sp.	B18	culled onion from storage NY, USA	this study	MK391693
Rahnella sp.	C1b	culled onion from storage NY, USA	this study	MK391694
Rahnella sp.	C10	culled onion from storage NY, USA	this study	MK408759
Rahnella sp.	D36	culled onion from storage NY, USA	this study	MK391695
Rahnella sp.	E32Ma	culled onion from storage NY, USA	this study	MK391696
Rahnella sp.	F30a	culled onion from storage NY, USA	this study	MK391697

Rahnella sp.	F35b	culled onion from storage NY, USA	this study	MK391698
Rahnella sp.	F57b	culled onion from storage NY, USA	this study	MK391699
Rahnella sp.	FC61912-K	creek water, NY, USA	this study	MK391700
Rahnella sp.	G4	culled onion from storage NY, USA	this study	MK391701
Rahnella sp.	G29a	culled onion from storage NY, USA	this study	MK391703
Rahnella sp.	G33b	culled onion from storage NY, USA	this study	MK391704
Rahnella sp.	G37d	culled onion from storage NY, USA	this study	MK391705
Rahnella sp.	G42	culled onion from storage NY, USA	this study	MK391702
Rahnella sp.	G43a1	culled onion from storage NY, USA	this study	MK391706
Rahnella sp.	H11b	culled onion from storage NY, USA	this study	MK391707
Rahnella sp.	H23	culled onion from storage NY, USA	this study	MK391708
Rahnella sp.	I50b	freshly harvested symptomatic onion bulb NY, USA	this study	MK391709
Rahnella sp.	J9a	non-symptomatic yellow onion from storage NY, USA	this study	MK391710
Rahnella sp.	J55b	non-symptomatic yellow onion from storage NY, USA	this study	MK391711
Rahnella sp.	K60d	symptomatic red onion from storage NY, USA	this study	MK391712
Rahnella sp.	L50a	symptomatic red onion from storage NY, USA	this study	MK391713
Rahnella sp.	L52e	symptomatic red onion from storage NY, USA	this study	MK391714
Rahnella sp.	L54a	symptomatic yellow onion from storage NY, USA	this study	MK391715
Rahnella sp.	L70b	symptomatic yellow onion from storage NY, USA	this study	MK391716
Rahnella sp.	L72c	symptomatic yellow onion from storage NY, USA	this study	MK391717
Rahnella sp.	N27b	non-symptomatic yellow onion from storage NY, USA	this study	MK391718

Rahnella sp.	N81a	symptomatic yellow onion from storage NY, USA	this study	MK391719
Rahnella sp.	N89b	symptomatic yellow onion from storage NY, USA	this study	MK391720
Rahnella sp.	L18a	symptomatic yellow onion from storage NY, USA	this study	MK391721
Rahnella sp.	AG1a	symptomatic onion transplant, NY USA	this study	MK391722
Rahnella sp.	P36c	symptomatic red onion bulb from storage NY, USA	this study	MK391723
Rahnella sp.	AP10b	symptomatic onion plant NY, USA	this study	MK391724
Rahnella sp.	AP11b	symptomatic onion plant NY, USA	this study	MK391725
Rahnella sp.	AR16b	symptomatic onion bulb grown in OR, USA	this study	MK391726
Rahnella sp.	C60	symptomatic red onion bulb from storage NY, USA	this study	MK391727
Rahnella sp.	C81a	symptomatic red onion bulb from storage NY, USA	this study	MK391728
Rahnella sp.	L31-1-12	onion from county of Vestfold, Norway	this study	MK391741
Rahnella sp.	L57-1-12	onion from county of Oppland, Norway	this study	MK391742
Rahnella sp.	L151-1a	onion from county of Østfold, Norway	this study	MK391743
Rahnella sp.	L172-1A	onion from county of Vestfold, Norway	this study	MK391744
Rahnella sp.	L173-1B	onion from county of Vestfold, Norway	this study	MK391745
Rahnella sp.	SL6	onion from county of Hedmark, Norway	this study	MK391746
Rahnella aquatilis	ATCC 33071	drinking water, France (type strain)	Martinez *et al*. (2012a)	CP003244.1
Rahnella victoriana	USA13	*Quercus kelloggii*, symptomatic inner bark, California, USA	Brady *et al.* (2014)	MK391729
Rahnella bruchi	ALN 45	*Alnus glutinosa*, inner bark, Surrey, UK	Brady *et al.* (2014)	MK391730
Rahnella bruchi	FRB 226^{T}	*Agrilus biguttatus*, gut, Shropshire, UK	Brady *et al.* (2014)	MK391740
Rahnella woolbedinensis	FRB 227	*Alnus glutinosa,* inner bark, Surrey, UK	Brady *et al.* (2014)	MK391731

Rahnella woolbedingensis	WAL 10	*Juglans regia*, inner bark, Surrey, UK	Brady *et al.* (2014)	MK391732
Ewingella americana	AT 14b	*Quercus robur*, symptomatic inner bark, Shropshire, UK	Brady *et al.* (2014)	MK391733
Ewingella americana	FOD 24/3b	*Quercus robur,* symptomatic inner bark, Gloucestershire, UK	Brady *et al.* (2014)	MK391735
Ewingella americana	ATCC 33852	human throat, KY, USA (type strain)		JMPJ01000067.1
Rahnella victoriana	FRB 225	*Quercus robor*, symptomatic inner bark, Suffolk, UK	Brady *et al.* (2014)	MK391736
Rahnella variigena	FOD 20/8	*Quercus robur*, wound response fluid, Gloucestershire, UK	Brady *et al.* (2014)	MK391737
Rahnella variigena	PFK 1/1C2a	*Quercus robur*, symptomatic inner bark, Sussex, UK	Brady *et al.* (2014)	MK391738
Rahnella inusitata	FOD 9/21	*Quercus robur,* symptomatic inner bark, Gloucestershire, UK	Brady *et al.* (2014)	MK391739
Rahnella inusitata	FOD 9/5a	*Quercus robur*, symptomatic inner bark, Gloucestershire, UK	Brady *et al.* (2014)	MK391734
Xenorhabdus nematophila	ATCC 19061			FN667742.1

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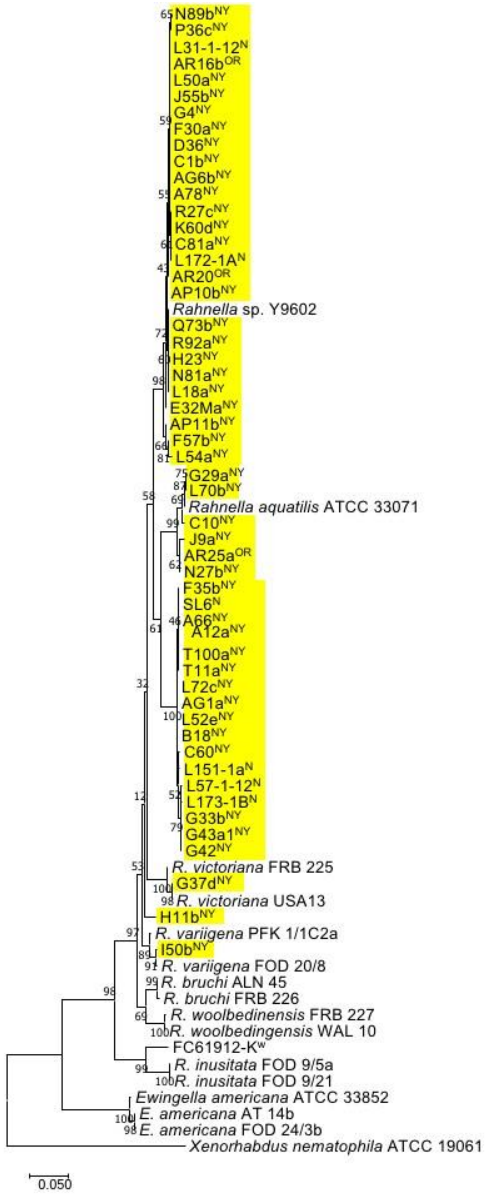
Table S2 Accession numbers for sequences used in multilocus sequence analysis

	Strain	Source	*gyrB*	*rpoB*	*infB*	*atpD*
Rahnella sp.	A66	culled onion from storage NY, USA	MK387415	MK387404	MK387393	MK387382
Rahnella sp.	AR25a	onion bulb grown in OR, USA	MK387416	MK387405	MK387394	MK387383
Rahnella sp.	C1b	culled onion from storage NY, USA	MK387417	MK387406	MK387395	MK387384
Rahnella sp.	F57b	culled onion from storage NY, USA	MK387418	MK387407	MK387396	MK387385
Rahnella sp.	FC61912-K	river water, NY, USA	MK387419	MK387408	MK387397	MK387386
Rahnella sp.	G37d	culled onion from storage NY, USA	MK387420	MK387409	MK387398	MK387387
Rahnella sp.	H11b	culled onion from storage NY, USA	MK387421	MK387410	MK387399	MK387388
Rahnella sp.	L31-1-12	onion from county of Vestfold, Norway	MK387422	MK387411	MK387400	MK387389
Rahnella sp.	L57-1-12	onion from county of Oppland, Norway	MK387423	MK387412	MK387401	MK387390
Rahnella sp.	L172-1A	onion from county of Vestfold, Norway	MK387424	MK387413	MK387402	MK387391
Rahnella sp.	SL6	onion from county of Hedmark, Norway	MK387425	MK387414	MK387403	MK387392

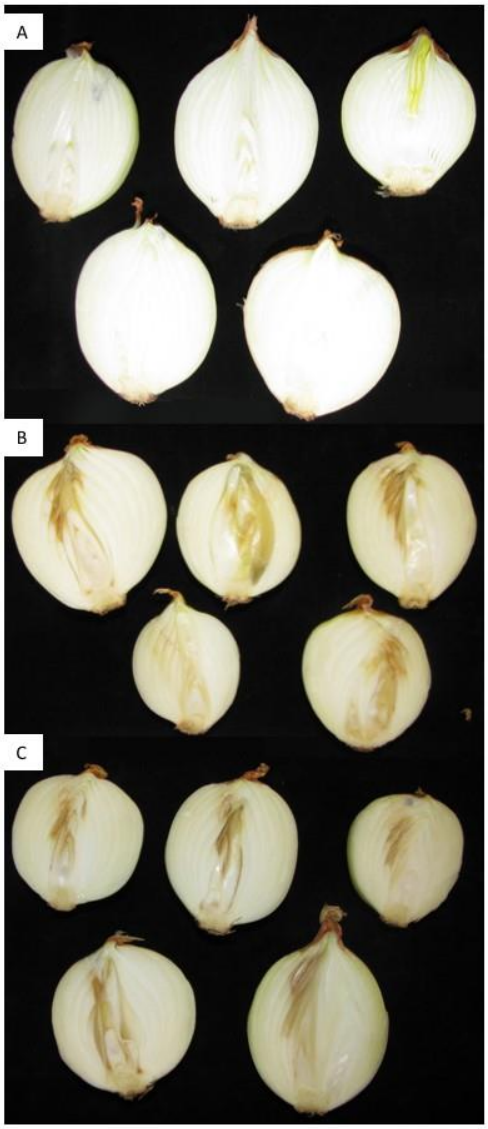
Table S3 Genera recovered from surveys of diseased and healthy onion bulbs in USA

	Cull Survey		Random Survey	
Total Number of Bulbs	508		898	
	Symptomatic	Non-Symptomatic	Symptomatic	Non-Symptomatic
Total Number of Bulbs	477	31	150	748
No bacteria isolated	63	21	12	564
Rahnella spp.	136	0	25	9
Enterobacter spp.	62	1	47	34
Pseudomonas spp.	138	2	53	28
Burkholderia spp.	52	0	31	77
Pantoea spp.	36	0	41	55
Bacteria co-isolated with *Rahnella*				
Nothing or multiple *Rahnella* strains	55	0	7	2
Unidentified bacteria only	26	0	1	2
Pseudomonas spp. only	23	0	7	0
Enterobacter spp. only	6	0	1	1
Pantoea spp. only	2	0	1	0
Burkholderia spp. only	1	0	0	2
Citrobacter sp.	0	0	2	0
Klebsiella sp.	0	0	1	0
Multiple other bacteria Isolated	5	0	5	2



190x254mm (96 x 96 DPI)

190x254mm (96 x 96 DPI)



190x254mm (96 x 96 DPI)