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1 Title;

2 Autumn potato seedling failure due to potato dry rot in Nagasaki Prefecture, Japan,  
3 caused by *Fusarium acuminatum* and *Fusarium commune*

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25 Footnotes;

26 The nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank  
27 databases under the accession numbers LC469781–LC469790.

28

29 Author contributions;

30 All authors contributed to the study conception and design. Material preparation, data  
31 collection and analysis were performed by Hisashi Osawa, Yu Sakamoto, Seishi Akino  
32 and Norio Kondo. The first draft of the manuscript was written by Hisashi Osawa and  
33 all authors commented on previous versions of the manuscript. All authors read and  
34 approved the final manuscript.

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49 Abstract

50 Failure to sprout due to seed-tuber rot is a serious problem for autumn potato

51 seedling cultivation in Nagasaki Prefecture, Japan. In this study, five strains were  
52 isolated from rotten seed tubers sampled in 2015; when tubers were inoculated with  
53 these strains, the tubers developed rot and failed to sprout. We identified these strains as  
54 *Fusarium acuminatum*, *Fusarium commune*, and the known agent of potato dry rot,  
55 *Fusarium oxysporum* based on morphological and DNA sequencing analyses. *F.*  
56 *commune* and *F. acuminatum* were identified as causal agents of potato dry for the first  
57 time in the world and in Japan, respectively.

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59 Keywords

60 Dry rot, Potato, *Fusarium commune*, *Fusarium oxysporum*, *Fusarium acuminatum*

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74 Double cropping of potatoes is common in parts of southwestern Japan, such  
75 as Nagasaki Prefecture in the Kyushu region, but seed-tuber rot has caused a shortage of

76 autumn seedlings (Sakamoto et al. 2016a), especially of cultivars Aiyutaka (Nakao et al.  
77 2004) and Sanjumaru (Mukojima et al. 2012) and yield losses in the main cropping  
78 areas of Nagasaki. Sections of rotten seed tubers were covered with mycelia and  
79 occasionally have wrinkles. Sprouting disorders have also been observed (Sakamoto et  
80 al. 2016b). Internal necrotic areas of rotten tubers were shades of brown, ranging from  
81 fawn to dark chocolate. Some severely rotten tubers may exhibit a soft rot caused by  
82 secondary saprophytic microorganisms. When viewed with a microscope, tuber sections  
83 contained hyphae with crescent-shaped conidia characteristic of *Fusarium* spp. Here we  
84 identified the causal agents of this tuber rot and sprouting disorder.

85 Rotten seed tubers were unearthed from areas where seedlings failed to appear  
86 in potato cropping fields of the Nagasaki Agricultural and Forestry Technical  
87 Development Center (Unzen, Nagasaki, Japan) in October 2015. The rotten tubers were  
88 placed in plastic containers with wet paper and incubated at room temperature for 2–7  
89 days. Newly produced hyphal masses were sampled and cultured on Komada medium  
90 (Komada 1975). Eighteen single-spore isolates were obtained from colonies grown on  
91 Komada medium, and five strains (F1501, F1503, F1510, F1516, and F1514) were  
92 selected from each host cultivar. Each isolate was grown on potato-sucrose agar  
93 medium for 2 weeks to obtain conidia. They were tested for pathogenicity by dipping  
94 six cut tubers (cv. Irish Cobbler) in a suspension of the respective conidia ( $2.0 \times 10^5$   
95 conidia/mL) for approximately 10 s. The tubers were then dried, placed in plastic bags  
96 filled with noninfested potting soil mixture (Katakura & Co-op Agri, Tokyo, Japan), and  
97 incubated at 25°C for 8 days. Tubers were then checked for rot. Tubers for a negative  
98 control were treated the same way but dipped in distilled water instead of a conidial  
99 suspension. All inoculated tubers had abundant mycelia on the surface (Fig. 1a), except  
100 for two tubers inoculated with F1516; and all tubers with mycelia were soft rot. Some

101 rotten tubers had symptoms similar to those of dry rot including wrinkles and gaps, in  
102 addition to internal lesions filled with mycelia. The rotten tissue caused by strain F1514  
103 was dark brown, whereas rotten tissue caused by other strains was cream-colored to  
104 light brown. Sprouting and root growth were observed in tubers in the negative control  
105 plot. However, inoculated tubers roots rarely grew; sprouting was not observed in any  
106 of the five tested strains (Fig. 1a, b). The five pathogenic strains (F1501, F1503, F1510,  
107 F1516, and F1514) were provided to the NARO Genebank project, Japan, as  
108 MAFF246882–246886, respectively.

109 DNA was extracted from approximately 100 mg of fresh mycelia, that had  
110 been grown in potato-sucrose broth for 2 weeks, using a FavorPrep Plant Genomic  
111 DNA Extraction Mini Kit (Favorgen Biotech, Ping-Tung, Taiwan). Translation  
112 elongation factor 1- $\alpha$  (TEF) (O'Donnell et al. 1998) and mitochondrial small subunit  
113 (mtSSU) rDNA regions (White et al. 1990) were amplified by PCR in a 25- $\mu$ L reaction  
114 volume containing 1  $\mu$ L template DNA, 15  $\mu$ L distilled water, 2.5  $\mu$ L 10 $\times$  Ex Taq buffer,  
115 2.0  $\mu$ L 2.5 mM dNTPs, 1.6  $\mu$ L 25 mM MgCl<sub>2</sub>, 2.5  $\mu$ L 0.1% (w/v) bovine serum  
116 albumin, 0.4 U TaKaRa Ex *Taq* polymerase (TaKaRa Bio, Kusatsu, Japan), and 10  $\mu$ M  
117 of each primer as previously described (O'Donnell et al. 1998; White et al. 1990). The  
118 thermal cycling conditions for TEF were initial denaturation at 95°C for 5 min; 36  
119 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at  
120 72°C for 1 min; and final extension at 72°C for 10 min. For mtSSU, the conditions were  
121 the same as for TEF except for annealing at 50°C. PCR products were purified using a  
122 NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany) and sequenced  
123 by a DNA sequencing service (Hokkaido System Science, Sapporo, Japan). Sequence  
124 data were aligned using ClustalW software (Thompson et al. 1994), and DNA  
125 sequences of closely related strains were obtained from GenBank and NARO Genebank.

126 Aligned sequences from TEF and mtSSU **were connected**, and neighbor-joining  
127 phylogenetic trees were produced using the Kimura-2 parameter model (Kimura 1980) in  
128 MEGA7 (Kumar et al. 2016). Bootstrap values were determined from 1,000 replications  
129 of the calculations. All gaps and missing data were eliminated from the data sets.  
130 According to a phylogenetic tree from the combined TEF and mtSSU data, the strains  
131 cluster into three clades: F1501 belongs to the clade of *Fusarium commune*; F1503,  
132 F1510, and F1516 to the clade of *Fusarium oxysporum*; and F1514 to the clade of  
133 *Fusarium acuminatum* (Fig. 2). The DNA sequences of the five tested strains were  
134 submitted to the DNA Data Bank of Japan (DDBJ), European Molecular Biology  
135 Laboratory (EMBL), and GenBank as accession numbers LC469781–LC469790.

136 For morphological observations, the five strains were cultured on potato  
137 dextrose agar (PDA) at 25°C **in darkness for 2 weeks**. Colony morphologies were then  
138 examined. **Structural characteristics** of the strains (e.g., conidia, chlamydo-spores, and  
139 phialides) were observed in cultures grown on synthetic low-nutrient agar (SNA)  
140 (Nirenberg and O'Donnell 1998) at 25°C under continuous black light for 2 weeks.  
141 **Conidia** and chlamydo-spores ( $n = 50$  each) were measured to determine means and  
142 ranges. The microconidia of all strains were aseptate or 1-septate, and oval or reniform  
143 in shape. The conidia formed false heads on monophialides from short conidiophores.  
144 Considerable differences in morphological characteristics were observed between  
145 F1514 and the other strains. Colony growth of F1514 **on PDA** was slower and the  
146 underside of the colonies was carmine-colored, whereas the others lacked color or were  
147 purple or violet. The macroconidia produced by F1514 **on SNA** were generally 4–  
148 5-septate, equilaterally curved, and slender, with distinct foot-shaped basal cells. The  
149 other strains formed mainly micro- and macroconidia. The macroconidia were generally  
150 3–4-septate and moderately curved, with foot-shaped basal cells (Table S1, Fig. 3). The

151 teleomorph stage was not observed for any tested strains grown on SNA or PDA. The  
152 morphological characteristics of all fungal isolates were compared with published  
153 descriptions (Gerlach and Nirenberg 1982). F1514 and others (1503, F1510, and F1516)  
154 had characteristics similar to those of *F. acuminatum* and *F. oxysporum*, respectively.  
155 The characteristics of F1501, which was in the *F. commune* clade, were also similar to  
156 those reported for *F. commune* (Skovgaard et al. 2003).

157         The results of this study suggest that the lack of sprouting of autumn potato  
158 seedlings in Nagasaki was attributable to potato dry rot caused by *Fusarium* spp., such  
159 as *F. acuminatum*, *F. commune*, and *F. oxysporum*. A new taxonomic classification and  
160 name for *F. oxysporum* have been proposed (Lombard et al. 2019); however, we  
161 adopted the previous classification in this study because the new name remains  
162 controversial. Initially, inoculated tubers were covered with mycelia, had dry rot,  
163 wrinkles, and voids with internal lesions. In Japan, *Fusarium avenaceum*, *F. oxysporum*,  
164 *Fusarium solani* f. sp. *eumartii*, *F. solani* f. sp. *radicicola*, *Fusarium caeruleum*,  
165 *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium ventricosum*, and *Fusarium*  
166 *sambucinum* have been identified as causal agents of potato dry rot (Kodama 2004).  
167 However, the present study is the first to report potato dry rot caused by *F. commune*  
168 and *F. acuminatum* globally and in Japan, respectively. *F. commune* has been identified  
169 as a causal agent of root rot and damping-off in forest nurseries (Kim et al. 2012;  
170 Stewart et al. 2006); damping-off, seed rot, and seedling root rot in soybeans (Ellis et al.  
171 2013); and crown and root rot in tomatoes (Hamini-Kadar et al. 2010). Both *F.*  
172 *commune* and *F. acuminatum*, reported here, may be indigenous to Japan because  
173 imported seed tubers have not been grown in the fields surveyed.

174

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180

181 Compliance with ethical standards

182 Conflict of interest: The authors declare that they have no conflict of interest.

183 Ethical approval: This article does not contain any studies with human participants or  
184 animals performed by any of the authors.

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246 Figure legends

247 **Fig. 1** Cut tubers (a) inoculated with pathogenic *Fusarium* strain F1510 or (b) Cut  
248 tubers inoculated with distilled water as negative control.

249

250 **Fig. 2** Neighbor-joining phylogenetic tree based on translation elongation factor 1- $\alpha$  and

251 mitochondrial small subunit rDNA sequences. Numbers on branches indicate bootstrap  
252 values obtained for 1,000 replicates. Bars indicate substitutions per site. (a) F1501,  
253 F1503, F1510, F1516, and related species. T, ex-type strain. (b) F1514 and related  
254 species.

255

256 **Fig. 3** Morphological characteristics of isolated strains. (a–c) Colonies on  
257 potato-dextrose agar at 25°C after 2 weeks in darkness. (a–c) Left half of plate images,  
258 top surface of plate and right half, reverse side of isolate (a) F1501 (*Fusarium*  
259 *commune*), (b) F1510 (*Fusarium oxysporum*), and (c) F1514 (*Fusarium acuminatum*).  
260 (d–f) Macroconidia of (d) F1501 (*F. commune*), (e) F1516 (*F. oxysporum*), and (f)  
261 F1514 (*F. acuminatum*). (g) Short conidiophores and microconidia produced false heads  
262 with monophialidic phialides (F1516: *F. oxysporum*). (h) Smooth-walled  
263 chlamydospores (F1501: *F. commune*). (i) Oval or reniform-shaped microconidia  
264 (F1503: *F. oxysporum*). Scale bars, 20 µm.

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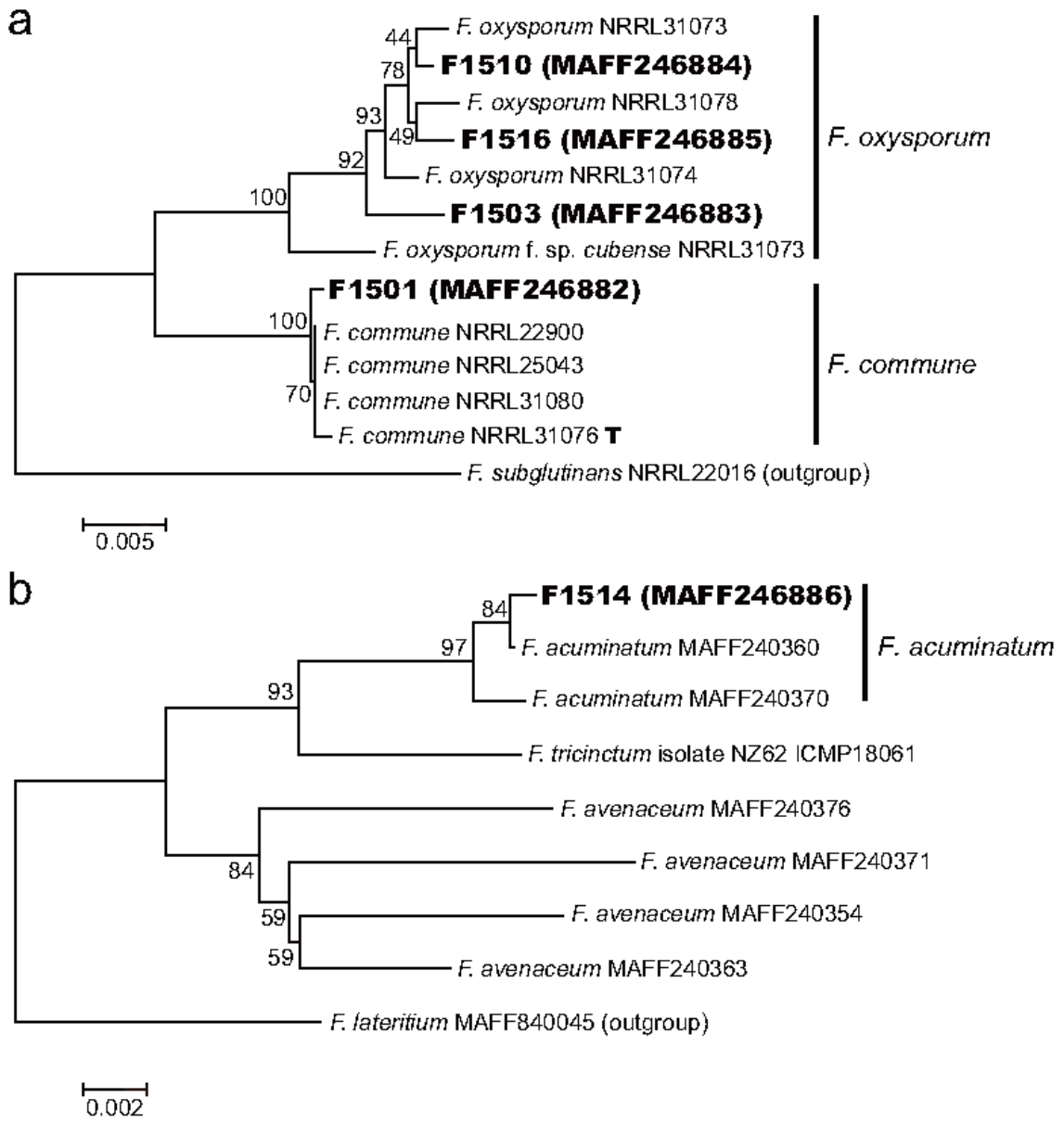
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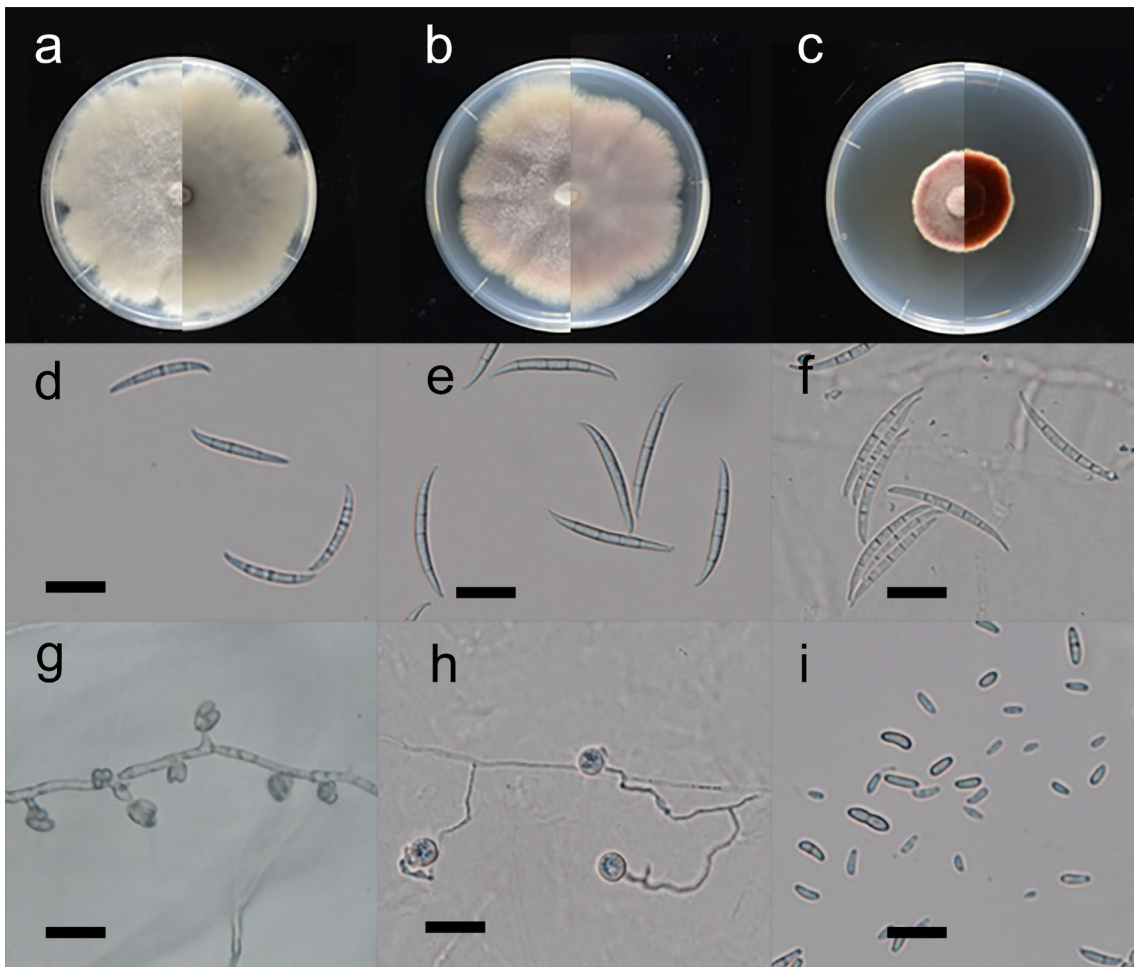
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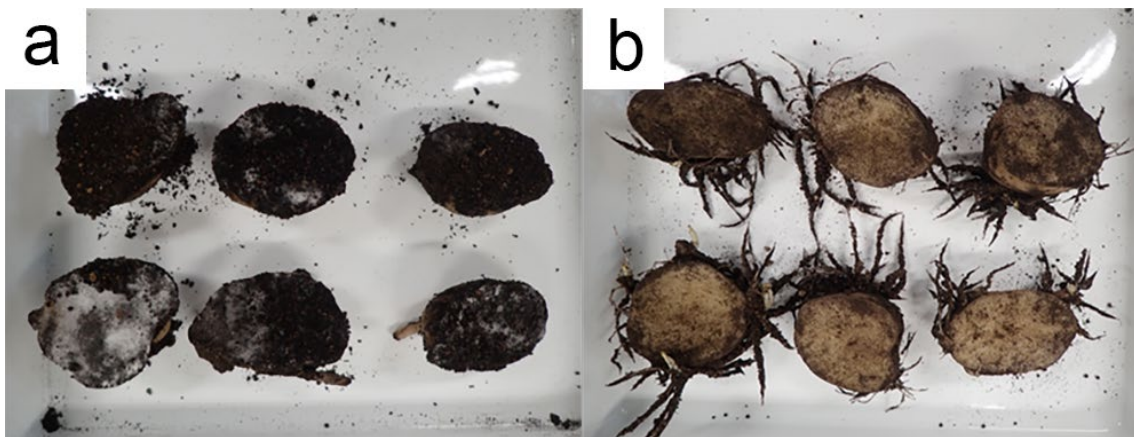
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