Title;

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

Authors;

Hisashi Osawa, Yu Sakamoto, Seishi Akino and Norio Kondo

The affiliations and addresses of the authors;

Hisashi Osawa (ORCID ID: 0000-0003-1726-5796)

Graduate School of Agriculture, Hokkaido University, Kita-ku Kita 9 Nishi 9, Sapporo, 060-8589, Japan

Yu Sakamoto

Nagasaki Agricultural and Forestry Technical Development Center, 2777, Otsu, Aino-cho, Unzen, Nagasaki, 854-0302, Japan

Seishi Akino (ORCID ID: 0000-0002-6864-4684), Norio Kondo

Research Faculty of Agriculture, Hokkaido University, Kita-ku Kita 9 Nishi 9, Sapporo, 060-8589, Japan

Corresponding author;

Hisashi Osawa, email: hi_osawa@frontier.hokucdai.ac.jp, TEL 011-706-2488

Total text pages: 11

The number of tables and figures: 4 (3 figures and 1 supplementary table)

Footnotes;

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

Author contributions;

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

Abstract

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

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

Keywords

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

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

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

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

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

DNA was extracted from approximately 100 mg of fresh mycelia, that had been grown in potato-sucrose broth for 2 weeks, using a FavorPrep Plant Genomic DNA Extraction Mini Kit (Favorgen Biotech, Ping-Tung, Taiwan). Translation elongation factor 1-α (TEF) (O'Donnell et al. 1998) and mitochondrial small subunit (mtSSU) rDNA regions (White et al. 1990) were amplified by PCR in a 25-μL reaction volume containing 1 μL template DNA, 15 μL distilled water, 2.5 μL 10× Ex Taq buffer, 2.0 μL 2.5 mM dNTPs, 1.6 μL 25 mM $MgCl_2$, 2.5 μL 0.1% (w/v) bovine serum albumin, 0.4 U TaKaRa Ex *Taq* polymerase (TaKaRa Bio, Kusatsu, Japan), and 10 μM of each primer as previously described (O'Donnell et al. 1998; White et al. 1990). The thermal cycling conditions for TEF were initial denaturation at 95°C for 5 min; 36 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min; and final extension at 72°C for 10 min. For mtSSU, the conditions were the same as for TEF except for annealing at 50°C. PCR products were purified using a NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany) and sequenced by a DNA sequencing service (Hokkaido System Science, Sapporo, Japan). Sequence data were aligned using ClustalW software (Thompson et al. 1994), and DNA sequences of closely related strains were obtained from GenBank and NARO Genebank.

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

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

175 Acknowledgments

The authors thank Dr. Takayuki Aoki (National Agricultural and Food Research Organization) and Dr. Tomoo Misawa (Hokkaido Research Organization) for useful advice regarding phylogenic analyses, and Mr. Yoshinori Hirayama for data collection assistance.

Compliance with ethical standards

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

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

References

Ellis ML, Arias MMD, Jimenez DRC, Munkvold GP, Leandro LF (2013) First report of *Fusarium commune* causing damping-off, seed rot, and seedling root rot on soybean (*Glycine max*) in the United States. Plant Dis 97:284

Gerlach W, Nirenberg H (1982) The genus *Fusarium*—a pictorial atlas. Mitt Biol Bundesanst Land-u Forstw, Berlin-Dahlem, Germany

Hamini-Kadar N, Edel-Hermann V, Gautheron N, Steinberg C (2010) First report of *Fusarium commune* and *Fusarium redolens* causing crown and root rot on tomato in Algeria. New Dis Rep 22:3

Kim MS, Stewart JE, Dumroese RK, Klopfenstein NB (2012) Occurrence of the root rot pathogen, *Fusarium commune*, in forest nurseries of the midwestern and western United States. J Phytopathol 160:112–114

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

201 Kodama F (ed) (2004) Manual of pest control in Hokkaido (in Japanese). Hokkaido
202 Plant Protect Assoc, Sapporo, Japan
203 Komada H (1975) Development of a selective medium for quantitative isolation of
204 *Fusarium oxysporum* from natural soil. Rev Plant Protec Res 8:114–125
205 Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics
206 analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
207 Lombard L, Sandoval-Denis M, Lamprecht SC, Crous PW (2019) Epitypification of
208 *Fusarium oxysporum*–clearing the taxonomic chaos. Persoonia 43:1–47
209 Mukojima N, Mori K, Sakamoto Y, Tamiya S, Sohbaru N, Ishibashi Y, Nakao T (2012)
210 A new potato variety "Sanjumaru" (in Japanese with English summary). Bull
211 Nagasaki Agric & Forest Tech Dev Cen 3:27–51
212 Nakao T, Mukoujima N, Mori K, Ishibashi Y, Chaya M, Mori M (2004) A new potato
213 variety "Aiyutaka" (in Japanese with English summary). Bull Nagasaki Agric &
214 Forest Exp Station Sect Agric 30:1–28
215 Nirenberg HI, O'Donnell K (1998) New *Fusarium* species and combinations within the
216 *Gibberella fujikuroi* species complex. Mycologia 90:434–458
217 O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins
218 of the fungus causing Panama disease of banana: concordant evidence from
219 nuclear and mitochondrial gene genealogies. Proc Natl Acad Sci USA 95:2044–
220 2049
221 Sakamoto Y, Mori K, Watanabe W, Matsuo Y, Ozaki T, Nakao T (2016a) Technology
222 for stable germination of a potato variety "Sanjumaru" in fall cropping (The 1st
223 report: Promotion measure of dormancy breaking by a storage condition of seed
224 tubers) (in Japanese). Rep Kyushu Br Crop Sci Soc Japan 82:19–22
225 Sakamoto Y, Mori K, Watanabe W, Matsuo Y, Ozaki T, Nakao T (2016b) Technology

for stable germination of a potato variety “Sanjumaru” in fall cropping (The 2nd report: The rotting relief coutermeasures by the drying treatment of the surface of cutting seed tubers) (in Japanese). Rep Kyushu Br Crop Sci Soc Japan 82:23–26

Skovgaard K, Rosendahl S, O’Donnell K, Nirenberg HI (2003) *Fusarium commune* is a new species identified by morphological and molecular phylogenetic data. Mycologia 95:630–636

Stewart JE, Kim MS, James RL, Dumroese RK, Klopfenstein NB (2006) Molecular characterization of *Fusarium oxysporum* and *Fusarium commune* isolates from a conifer nursery. Phytopathology 96:1124–1133

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

White TJ, Bruns S, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Snisky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, CA, USA, pp 315–322

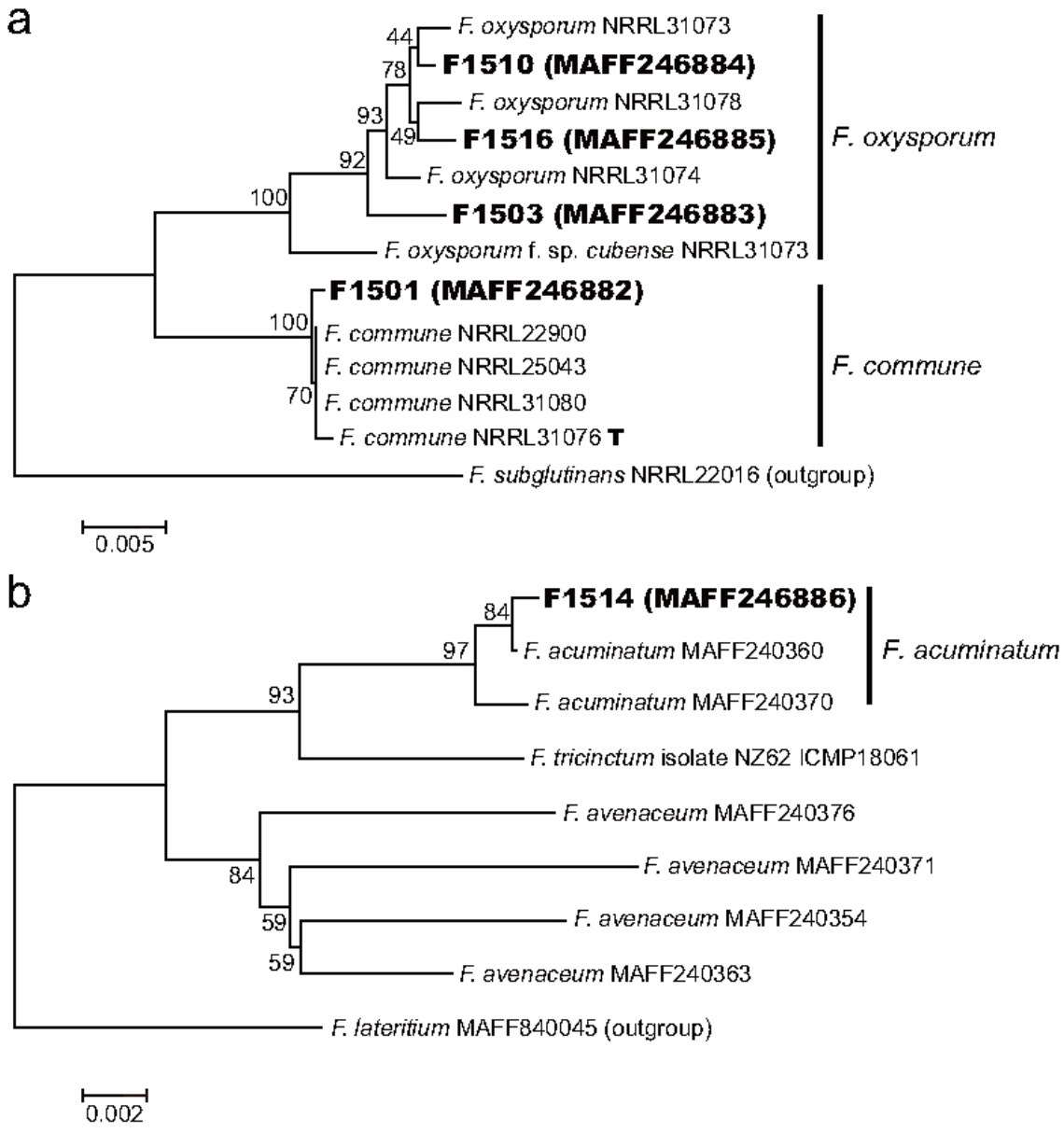
Figure legends

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

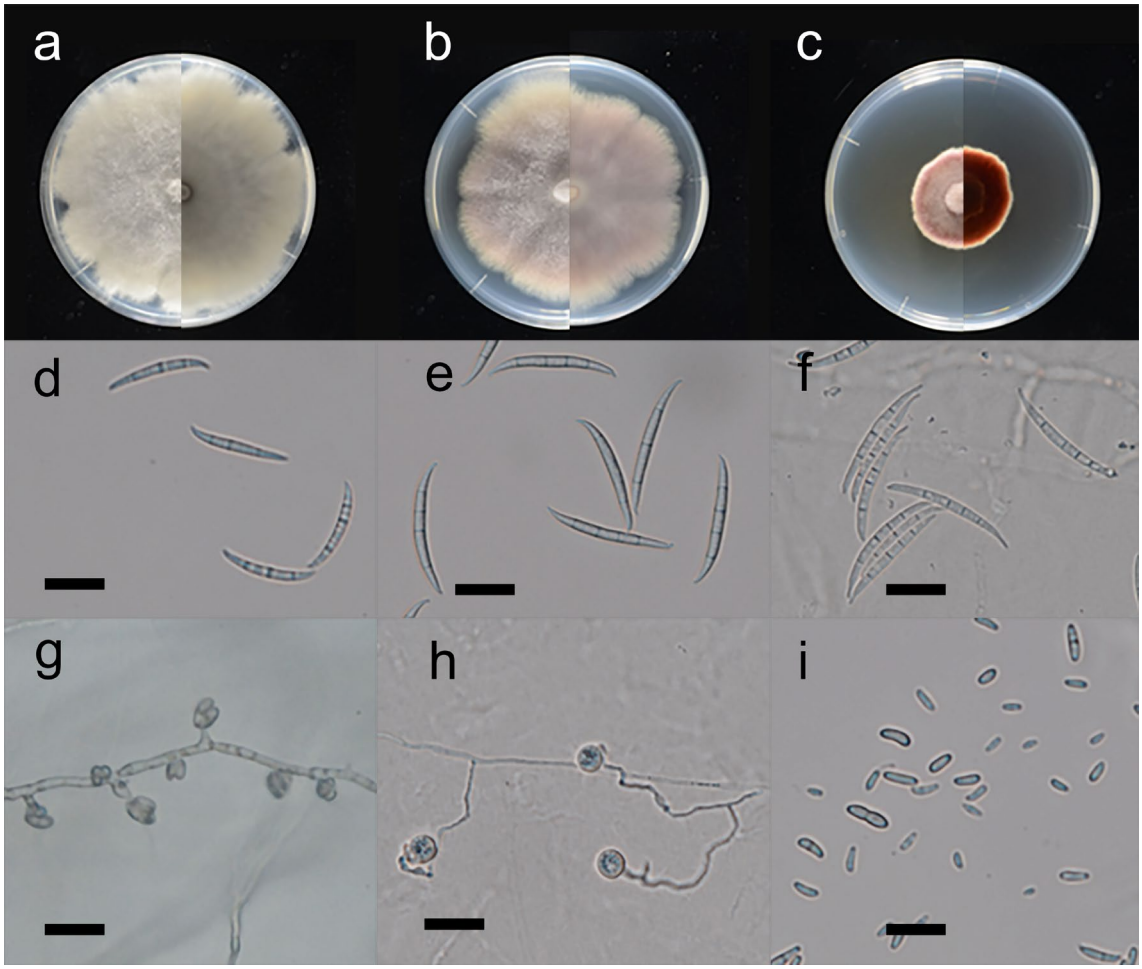
Fig. 2 Neighbor-joining phylogenic tree based on translation elongation factor 1-α and

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

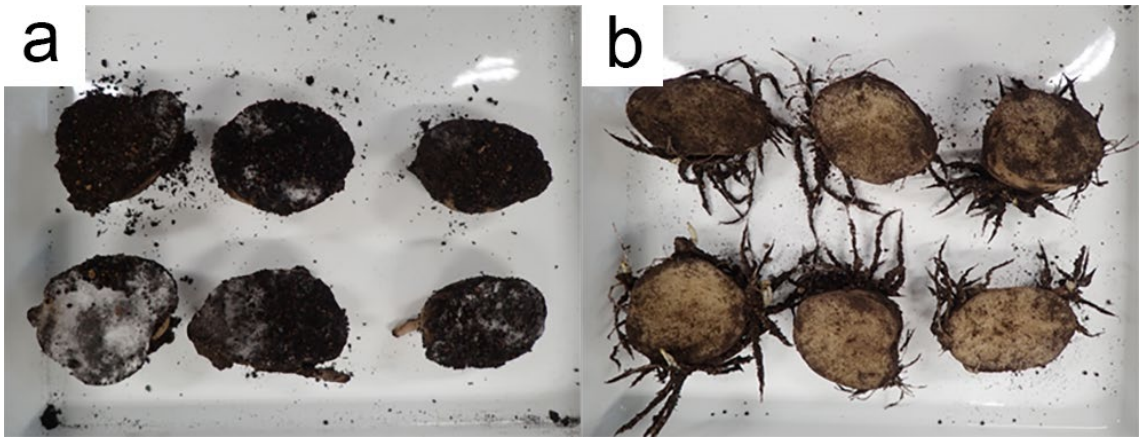
Fig. 3 Morphological characteristics of isolated strains. (a–c) Colonies on potato-dextrose agar at 25°C after 2 weeks in darkness. (a–c) Left half of plate images, top surface of plate and right half, reverse side of isolate (a) F1501 (*Fusarium commune*), (b) F1510 (*Fusarium oxysporum*),and (c) F1514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


a
F. oxysporum NRRL31073
F1510 (MAFF246884)
F. oxysporum NRRL31078
F1516 (MAFF246885)
F. oxysporum NRRL31074
F1503 (MAFF246883)
F. oxysporum f. sp. cubense NRRL31073
F. oxysporum
F1501 (MAFF246882)
F. commune NRRL22900
F. commune NRRL25043
F. commune NRRL31080
F. commune NRRL31076 T
F. commune
F. subglutinans NRRL22016 (outgroup)
44
78
93
49
92
100
100
70
0.005
b
F1514 (MAFF246886)
F. acuminatum MAFF240360
F. acuminatum MAFF240370
F. acuminatum
F. tricinctum isolate NZ62 ICMP18061
F. avenaceum MAFF240376
F. avenaceum MAFF240371
F. avenaceum MAFF240354
F. avenaceum MAFF240363
F. lateritium MAFF840045 (outgroup)
84
97
93
84
59
59
0.002

272



273



274