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Short communication

Genetic variation among natural isolates of the ectomycorrhizal hypogenous fungus, *Rhizopogon roseolus* from Japanese pine forests inferred using AFLP markers

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ABSTRACT

Genetic variation among 45 *Rhizopogon roseolus* isolates from 21 different regions of Japan were inferred using amplified fragment length polymorphism (AFLP) markers. Using three primer pair combinations, AFLP analysis reproducibly produced a total of 223 DNA fragments, 74.4% of which were polymorphic. Pairwise dissimilarity of AFLP patterns between isolates ranged from 0.043 to 0.228. Cluster analysis and principal coordinate analysis of AFLP data generally showed four major clusters from geographically distinct areas. The findings suggested that the Japanese populations of *R. roseolus* from different geographical regions can be distinguished based on AFLP characters.

The basidiomycete *Rhizopogon roseolus* (Corda) Th. Fr. (= *Rhizopogon rubescens* (Tul. & C. Tul.) Tul. & C. Tul.), an ectomycorrhizal (ECM) hypogeous fungus that forms globose to subglobose fruiting bodies underground, is primarily associated with the members of the Pinaceae (Molina et al. 1999). In Japan, *R. roseolus*, or "Shoro" as it is known, is regarded as an excellent edible fungus that grows in Japanese black pine (*Pinus thunbergii*) forests along the coast (Imazeki and Hongo 1989). In response to a marked decrease in annual production, fruiting bodies of *R. roseolus* command a high price in local markets. Attempts to cultivate this fungus in its natural habitat have been developed with methods of improving the forest environment or planting pine seedlings that had been artificially inoculated with *R. roseolus* ectomycorrhizae (Nagasawa 2000). However, the implementation of these methods has not yet been able to increase the supply of fruiting bodies to the market. In addition to developing a method for germinating *R. roseolus* basidiospores, Kawai et al. (2008) recently revealed that this species employs a bipolar mating system. Their findings are very interesting as they open the possibility of crossbreeding *R. roseolus* cultivars. In such crossbreeding experiments, information on genetic relatedness among the natural isolates of this fungus in Japan will be very useful in the evaluation of *R. roseolus* genetic resources.

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Molecular approaches are considered to be powerful tools for investigating the inter- and intra-genetic relatedness among basidiomycete species. Indeed, such molecular data can be used in conjunction with conventional methods employing morphological, physiological and biochemical characters. The amplified fragment length polymorphism (AFLP) technique combines both classical, hybridization-based fingerprinting methods with a PCR-based fingerprinting method (Vos et al. 1995). This technique is highly sensitive and reproducible along with the ability to detect a large number of loci covering a whole genome in a single assay, so it has been used in genetic study of many organisms

Table 1 – *Rhizopogon roseolus* isolates from Japan used in this study.

Isolate	Geographic location	Host	Year/month	Origin (Original description)	Accession no.
Iwate-1	Rikuzentakata-shi, Iwate	P. t	2003/07	Miyagi Pref. (30–41)	AB685718
Miyagi-1	Naruse-cho, Miyagi	P. t	1998/05	Miyagi Pref. (30–15)	AB685719
Miyagi-2	Sendai-shi, Miyagi	P. t	2003/07	Miyagi Pref. (30–42)	AB685386
Ibaraki-1	Asahi-son, Ibaraki	P. t	1999/04	Ibaraki Pref. (AT678)	AB685720
Ibaraki-2	Naka-shi, Ibaraki	P. d	1998/04	Ibaraki Pref. (AT632)	GQ179956
Ibaraki-3	Naka-shi, Ibaraki	P. t	–	NITE (33151)	NITE[a]
Ibaraki-4	Kashima-shi, Ibaraki,	P. t	1998/03	Ibaraki Pref. (AT630)	GQ17955
Niigata-1	Nakajo-cho, Niigata	P. t	2002/-	FFPRI (RR02Ni)	AB685721
Niigata-2	Ryozu-shi, Niigata	P. t	2005/11	Niigata Pref. (Nsado2)	AB685387
Niigata-3	Ryozu-shi, Niigata	P. t	2005/11	Niigata Pref. (Nsado3)	AB685388
Niigata-4	Ryozu-shi, Niigata	P. t	2005/11	Niigata Pref. (Nsado4)	AB685389
Niigata-5	Ryozu-shi, Niigata	P. t	2005/11	Niigata Pref. (Nsado5)	AB685390
Ishikawa-1	Nomi-shi, Ishikawa	P. t	2005/11	Ishikawa Pref. (C)	AB685722
Shizuoka-1	Kakegawa-shi, Shizuoka	P. t	2002/11	Shizuoka Pref. (1)	AB685723
Kyoto-1	Amino-cho, Kyoto	P. t	–	NITE (32812)	NITE[a]
Tottori-1	Tottori-shi, Tottori	P. t	1991/11	TMIC (31952)	AB685391
Tottori-2	Tottori-shi, Tottori	P. t	1991/11	TMIC (31956)	AB685392
Tottori-3	Tottori-shi, Tottori	P. t	1992/04	TMIC (32018)	AB685393
Tottori-4	Tottori-shi, Tottori	P. t	1992/04	TMIC (31983)	AB685394
Tottori-5	Tottori-shi, Tottori	P. t	1992/04	TMIC (31984)	AB685395
Tottori-6	Tottori-shi, Tottori	P. t	1992/04	TMIC (31985)	AB685396
Tottori-7	Tottori-shi, Tottori	P. t	1992/11	TMIC (32770)	AB685397
Tottori-8	Tottori-shi, Tottori	P. t	2005/04	This study	AB685410
Tottori-9	Tottori-shi, Tottori	P. t	2005/04	This study	AB685411
Tottori-10	Tottori-shi, Tottori	P. t	2005/04	This study	AB274244
Tottori-11	Tottori-shi, Tottori	P. t	2005/04	This study	AB685412
Tottori-12	Tottori-shi, Tottori	P. t	2005/04	This study	AB685413
Shimane-1	Izumo-shi, Shimane	P. t	2002/04	Shimane Pref. (106)	AB685724
Shimane-2	Shinji-cho, Shimane	P. t	2001/04	Shimane Pref. (107)	AB685725
Yamaguchi-1	Hikari-shi, Yamaguchi	P. t	1988/04	Yamaguchi Pref. (1)	AB685726
Tokushima-1	Kainan-cho, Tokushima	P. t	1996/11	Tokushima Pref. (TSYO1)	AB685727
Tokushima-2	Kainan-cho, Tokushima	P. t	1997/04	Tokushima Pref. (OSATO1)	AB685398
Kochi-1	Oogata-cho, Kochi	P. t	–	Kochi Pref. (20710)	AB685399
Kochi-2	Oogata-cho, Kochi	P. t	–	Kochi Pref. (20711)	AB685400
Kochi-3	Oogata-cho, Kochi	P. t	–	Kochi Pref. (20712)	AB685401
Kochi-4	Oogata-cho, Kochi	P. t	1998/03	Kochi Pref. (20714)	AB685402
Saga-1	Karatsu-shi, Saga	P. t	–	Saga Pref. (1)	AB685403
Saga-2	Karatsu-shi, Saga	P. t	–	Saga Pref. (2)	AB685404
Saga-3	Karatsu-shi, Saga	P. t	–	Saga Pref. (4)	AB685405
Saga-4	Karatsu-shi, Saga	P. t	–	Saga Pref. (B)	AB685406
Saga-7	Karatsu-shi, Saga	P. t	2001/-	FFPRI (RR01N)	AB685728
Miyazaki-1	Miyazaki-shi, Miyazaki	P. t	2003/-	FFPRI (RR03H)	AB685729
Kagoshima-1	Hioki-shi, Kagoshima	P. t	2002/03	Ibaraki Pref. (KM8)	AB685407
Kagoshima-2	Hioki-shi, Kagoshima	P. t	1999/-	FFPRI (RR99E)	AB685408
Kagoshima-3	Kagoshima-shi, Kagoshima	P. t	2003/-	FFPRI (RR03S)	AB685409

P. d, *Pinus densiflora*; P. t, *Pinus thunbergii*. –, unknown. FFPRI, Forestry and Forest Products Research Institute Kyushu Research Center; Ibaraki Pref., Ibaraki Prefectural Forestry Research Institute; Ishikawa Pref., Ishikawa-Ken Forest Experiment Station; Kochi Pref., Kochi Information, Research and Training Center; Miyagi Pref., Miyagi Prefectural Forestry Research Institute; Niigata Pref., Niigata Forestry and Forest Products Research Institute; NITE, National Institute of Technology and Evaluation (Biological Resource Center); Saga Pref., Saga Prefectural Forest Experiment Station; Shimane Pref., Shimane Prefecture Mountainous Region Research Center; Shizuoka Pref., Shizuoka Prefecture Forestry and Forest Products Research Institute; TMIC, Tottori Mycological Society Culture Collection; Tokushima Pref., Tokushima Agriculture, Forestry and Fisheries Technology Support Center Forest and Forestry Research Institute; Yamaguchi Pref., Yamaguchi Prefectural Forestry Guidance Institute.

[a] http://www.nbrc.nite.go.jp/alphabet/03_R.html.