Constructing a new integrated genetic linkage map and mapping quantitative trait loci for vegetative mycelium growth rate in Lentinula edodes

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\textbf{A B S T R A C T}

The most saturated linkage map for \textit{Lentinula edodes} to date was constructed based on a monokaryotic population of 146 single spore isolates (SSIs) using sequence-related amplified polymorphism (SRAP), target region amplification polymorphism (TRAP), insertion–deletion (InDel) markers, and the mating-type loci. Five hundred and twenty-four markers were located on 13 linkage groups (LGs). The map spanned a total length of 1006.1 cM, with an average marker spacing of 2.0 cM. Quantitative trait loci (QTLs) mapping was utilized to uncover the loci regulating and controlling the vegetative mycelium growth rate on various synthetic media, and complex medium for commercial cultivation of \textit{L. edodes}. Two and 13 putative QTLs, identified respectively in the monokaryotic population and two testcross dikaryotic populations, were mapped on seven different LGs. Several vegetative mycelium growth rate-related QTLs uncovered here were clustered on LG4 (Qmgr1, Qdgr1, Qdgr2 and Qdgr9) and LG6 (Qdgr3, Qdgr4 and Qdgr5), implying the presence of main genomic areas responsible for growth rate regulation and control. The QTL hotspot region on LG4 was found to be in close proximity to the region containing the mating-type A (MAT-A) locus. Moreover, Qdgr2 on LG4 was detected on different media, contributing 8.07%–23.71% of the phenotypic variation. The present study provides essential information for QTL mapping and marker-assisted selection (MAS) in \textit{L. edodes}.

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\textbf{Introduction}

\textit{Lentinula edodes} (shiitake), an edible mushroom native to East Asia, is one of the most widely cultivated edible mushrooms in the world (Bruhn \textit{et al.} 2009). Despite being an important part of Asian cuisine culture, \textit{L. edodes} has been increasingly adopted in Western cooking (Turto \textit{et al.} 2008). Pharmacologically, \textit{L. edodes} is renowned for its antitumor and antiviral...
activities, and it is the first medicinal macrofungus to enter the realm of modern biotechnology (Bisen et al. 2010). As a lignin-decomposing fungus, L. edodes possesses a powerful ligninolytic enzyme system (Kwan et al. 2012) and a huge potential for the bioconversion of lignocellulosic wastes (Gaitán-Hernández et al. 2006; Philipoussis et al. 2007).

New and fine strains are crucial for a sustainable development of L. edodes production and marketing. L. edodes is a tetrapolar heterothallic basidiomycete. Two monokaryons of L. edodes with different mating alleles at both the A and B mating-type loci are able to fuse and generate a dikaryon which is distinguished by the presence of clamp connections. In traditional hybridizations, crosses between compatible monokaryons are required to generate a series of hybrid dikaryons, and genotypes could only be indirectly selected based on the phenotypes of those hybrids in subsequent systematic selections (Foulongne-Oriol et al. 2012). However, appropriate molecular markers could be used to select genotypes directly based on the linkage relationships between markers and target genes in marker-assisted selection (MAS). This approach could greatly increase the genetic improvement efficiency of L. edodes strains.

Most of the important economic and agronomic traits of L. edodes, such as yield and mycelium growth rate, are quantitative traits controlled by multiple genes or quantitative trait loci (QTLs). As a result, it is difficult to improve these traits genetically by traditional breeding methods. By contrast, the utilization of molecular markers, genetic map and subsequent QTL mapping is more powerful in dissecting the genetic architecture of quantitative traits. It is also useful for carrying out MAS, which could increase the precision and efficiency of subsequent screening in breeding programs (Foulongne-Oriol et al. 2011).

Genetic maps of several edible mushrooms, including Agaricus bisporus (Kerrigan et al. 1993; Callac et al. 1997; Moquet et al. 1999; Foulongne-Oriol et al. 2010, 2011), Pleurotus ostreatus (Larraya et al. 2000; Park et al. 2006; Sivilapova et al. 2012), Pleurotus pulmonarius (Okuda et al. 2009) and Pleurotus eryngii (Okuda et al. 2012), have been reported previously. For L. edodes, several molecular genetic maps are currently available. Using 32 single spore isolates (SSIs) of the dikaryotic strain L-54, Kwan & Xu (2002) constructed the first molecular genetic map of L. edodes. The map was based on random amplified polymorphic DNA (RAPD) markers and spanned a distance of 622.4 cM. Based on 95 SSIs, high-efficiency amplified fragment length polymorphism (AFLP) markers were also used to construct linkage maps of L. edodes (Terasshima et al. 2002, 2006). Moreover, a genetic map spanning 908.8 cM was generated by tetrad analysis using 92 basidiosporic strains (Miyazaki et al. 2008). Just recently, low-coverage resequencing was used to rapidly genotype 20 SSIs and construct a genetic linkage map of L. edodes (Au et al. 2013). However, all these genetic maps were constructed with a small mapping population (less than 100 SSIs) and a low marker density (less than 300 markers). Population size and marker density are two main factors affecting the number and effects of QTLs detected, as well as the accuracy and precision of QTL estimates (Yu et al. 2011). Therefore, it is necessary to construct a high-quality genetic map of L. edodes based on more markers and a larger mapping population for better QTL mapping.

Several types of molecular markers are usually utilized in genetic mapping of edible mushrooms. PCR-based AFLP markers have been widely used for their relatively high reproducibility and efficiency (Foulongne-Oriol 2012). Sequence-related amplified polymorphism (SRAP) (Li & Quiros 2001) generates PCR-based markers targeting open reading frames (ORFs). Improved from SRAP, target region amplification polymorphism (TRAP) uses known partial sequence of a candidate gene as a fixed primer (Hu & Vick 2003). Because of their simplicity, reliability, moderate throughput, and facile sequencing of selected bands (Lin et al. 2003), SRAP and TRAP have been widely used in genetic mapping of plants (Gao et al. 2007; Yu et al. 2007; Chu et al. 2008). However, they were used scarcely in edible mushrooms.

As a quantitative trait, mycelium growth rate has attracted tremendous interest from mycologists due to its correlation with yield, inhibitory ability against competitors, and sexual recognition system (Larraya et al. 2001, 2002; van der Nest et al. 2009; Sivilapova et al. 2012). In P. ostreatus, the relationship between mycelium growth rate and the mating-type A (MAT-A) gene has been reported in monokaryons, with statistical analysis revealing a higher growth rate in monokaryons bearing the A2 mating allele than those bearing the A1 allele (Larraya et al. 2001). The result was supported by the linkage found between a QTL controlling dikaryotic mycelium growth rate and the MAT-A locus (Sivilapova et al. 2012). For L. edodes, only one putative QTL for vegetative mycelium growth rate of dikaryons on the potato dextrose agar medium was detected previously. This QTL and the MAT-A locus were not mapped on the same linkage group (LG) (Miyazaki et al. 2008). Research on vegetative mycelium growth is still in its infant stage, and currently, related systematic studies are very limited on monokaryons and dikaryons growing on the synthetic and complex media. Promising loci for genetic improvement of vegetative mycelium growth rate remain elusive, and thus it is necessary to dissect the QTLs controlling the vegetative mycelium growth rate of L. edodes.

In this study, we employed a monokaryotic population of 146 SSIs and constructed a new molecular linkage map containing 524 loci of L. edodes. QTL analyses of vegetative growth rate on different media were also carried out using one monokaryotic population and two testcross dikaryotic populations. The objectives of our study were to: (1) construct a high-quality linkage map for further genetic research; and (2) detect QTLs controlling the vegetative growth rate in L. edodes.

Materials and methods

Fungal strains and populations

Monokaryotic strains L205-6 (mating-type: A1B1) and W1-26 (A2B2) of L. edodes were selected respectively from germinating single spore cultures (monokaryons) of the cultivated strains L205 and WX1. The hybrid strain L6-26 was generated from the pairing between L205-6 and W1-26. The monokaryotic population consisted of 146 SSIs from mature fruiting bodies of strain L6-26. Mating-types of the monokaryotic strains were determined as previously reported (Darmono & Burdsall 1992; Li et al. 2007). Two tester monokaryotic strains, 741-15 (A1B2) and 741-64 (A2B3), were obtained from protoclonal
the wild dikaryotic strain LeQC741S from Sichuan Province as previously described by Kawasumi et al. (1987). All the 146 SSIs in the monokaryotic mapping population were paired with the two tester strains 741-15 and 741-64 to produce test-cross dikaryotic populations LQ-15 and LQ-64, respectively. Both LQ-15 and LQ-64 contained 146 dikaryotic strains. L. edodes strains L205 (CCTCC AF 2013008) and LeQC741S (CCTCC AF 2013009) were deposited in the China Center for Type Culture Collection whereas strain WX1 (ACCC 50926) was deposited in the Agricultural Culture Collection of China.

In the construction of the linkage map, only the monokaryotic segregating population including 146 SSIs was used, while in QTL mapping, all the 146 SSIs were used for the monokaryotic growth rate analysis, and all the dikaryotic strains in LQ-15 and LQ-64 were used for dikaryotic growth rate analysis.

**DNA extraction**

Mycelia of the 146 SSIs were incubated in liquid medium of malt extract, yeast extract and glucose (MYG) (2 % malt extract, 2 % glucose, 0.1 % peptone, and 0.1 % yeast extract) at 25 °C and collected by filtering. Total genomic DNA was extracted from the freeze-dried mycelia using the cetyltrimethylammonium bromide (CTAB) procedure (Murray & Thompson 1980). DNA was then detected as previously described (Xiao et al. 2010) and was diluted to 50 ng/µL.

**Marker development**

**SRAP and TRAP markers**

SRAP analysis was performed with 56 (7 × 8) primer pairs (Supplemental Table S1). PCR reactions were performed in a total volume of 20 µL consisting of 2 µL 10 × PCR buffer, 50 ng template DNA, 3 mM dNTP mix, 8 µM forward primers, 8 µM reverse primers, 40 mM MgCl₂, and 1.75 U Taq polymerase (TaKaRa, Japan). PCR amplifications were carried out in a MyCycler Thermal Cycler (Bio-Rad, USA) under the following conditions: 94 °C for 5 min, 5 cycles (at 94 °C for 1 min, 35 °C for 1 min, and 72 °C for 1 min), then 35 cycles (at 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min), and finally 72 °C for 7 min.

The 43 TRAP primer pairs used in this study are listed in Supplemental Table S2. In the TRAP analysis, the PCR reaction mixture and the amplification program used were the same as those for the SRAP analysis except that there were 12.5 µM forward primers (fixed primers) and 3 µM reverse primers (random primers) in the 20 µL mixture.

**Insertion—deletion (InDel) markers**

A total of 15 polymorphic primer pairs were chosen via an initial screening of 25 InDel test primers (Supplemental Table S3) using DNA samples of L205-6 and W1-26 as templates. All PCR reactions were performed in 20 µL mixture containing 2 µL 10 × PCR buffer, 50 ng template DNA, 5 mM dNTP mix, 10 µM forward primers, 10 µM reverse primers, 40 mM MgCl₂, and 1 U Taq polymerase. PCR amplifications were performed at 94 °C for 3 min, 35 cycles (at 94 °C for 30 s, 60 °C for 45 s, 72 °C for 1 min), and finally at 72 °C for 10 min.

Amplified fragments of SRAP, TRAP and InDel markers were all detected by electrophoresis on 6 % denaturing acrylamide gels stained with silver nitrate solution. SRAP markers were named by the forward and reverse primers used, followed by the approximate size of bands in base pairs (e.g. ME1-EM4-250); TRAP markers by the forward primer and reverse primer numbers, followed by the size of the bands (e.g. baw28-2-80); and InDel markers by the InDel primer name (e.g. S502R/F).

**Linkage analysis and mapping**

At each locus, a genotype identical to L205-6 was marked as “a”, while that to W1-26 was marked as “b”. All markers were evaluated by the Chi-square test for deviation from the expected 1:1 ratio among the strains in the monokaryotic mapping population. JoinMap V3.0 (Van Ooijen & Voorrips 2001) was used for the linkage analysis. Data of the monokaryotic mapping population were coded by the HAP (haploid) model. Markers were classified into LGs based on the minimum likelihood of odds (LOD) score of 3.0. Kosambi’s mapping function (Kosambi 1943) was used for calculating the linkage relationships between markers, recombination rates and genetic distances. The genetic map was graphically exhibited using Mapchart (Voorrips 2002).

**Estimation of genome length, map coverage and marker distribution**

The expected genome length (Le) was estimated using two different methods. In the first method, 2σ (σ is the average marker spacing over the entire linkage map) were added to the length of each LG to account for terminal chromosome regions (Fishman et al. 2001). In the second method, the length of each LG was multiplied by (m+1)/(m−1), where m is the number of markers on each LG (Chakravarti et al. 1991). The two estimates were then averaged and used as the expected genome length. The theoretical map coverage was estimated using the equation c = 1−e−2πσm Le, where c is the proportion of the genome within d cM of a marker, n is the number of markers in the map and Le is the estimated genome length (Remington et al. 1999; van der Nest et al. 2009).

The marker distribution between different LGs was evaluated by comparing the actual marker density with expected values under a Poisson distribution. Assuming an identical marker density across all LGs, the expected number of markers in the i-th LG (Li) was calculated by the equation

\[ \lambda_i = m \times \frac{L_i}{\sum L_i} \times G_i \]

where m is the total number of markers and G is the length of LGi after adding 2σ (Remington et al. 1999; Foulalonge-Oriol et al. 2010). The marker distribution within each LG was tested by whether the observed genetic positions of the markers are under a uniform distribution or not, using the Kolmogorov–Smirnov test (Foulalonge-Oriol et al. 2010).

**Phenotype determination and QTL mapping**

Monokaryotic growth rates of the 146 SSIs in the mapping population were determined on the MYG medium as previously described (Gong et al. 2013). For the LQ-15 and LQ-64 populations, growth rates of all dikaryotic strains in both the MYG and the complete yeast medium (CYM: 2 % glucose, 2 % agar, 0.2 % peptone, 0.2 % yeast extract, 0.1 % K₂HPO₄, 0.05 %
MgSO₄·7H₂O, 0.046 % KH₂PO₄ were determined. The final growth rate was calculated as the radial extension of each mycelial colony per day (Olson 2006). To evaluate the dikaryotic growth rates on the medium for commercial cultivation of L. edodes, glass tubes (18 mm in diameter, 18 cm in length) containing 18 g of mixed sawdust (SD) medium (79 % hardwood sawdust, 20 % wheat bran, and 1 % gypsum; 60 % humidity) were utilized for both testcross dikaryotic populations. The inoculum was placed at the top of each tube, and the growth rate was determined as the ratio of the distance colonized by the mycelium (in millimeters) to the growth time (20 d after inoculation). Three repetitions were performed for all the above-mentioned samples. Both the monokaryotic and dikaryotic samples were incubated at 25 °C in the dark.

For each medium, the effects of genotype on vegetative mycelium growth rate were assessed by one-way analysis of variance (ANOVA). For the synthetic media (MYG and CYM), the effects of tester strains and media on dikaryotic growth rates were analyzed by two-way ANOVA. For the mixed sawdust medium, the effects of the tester strains on dikaryotic growth rates were evaluated by the paired sample t-test. The Pearson procedure was used for correlation analyses between all types of mycelium growth rates in the three different populations and media. SPSS (Statistical Package for the Social Sciences) version 17 (SPSS Inc., Chicago, IL, USA) was used for all the data analyses.

For QTL mapping, each phenotypic data set was input to WinQTLCart 2.5 (Zeng 1994; Wang et al. 2012), and composite interval mapping (CIM) was used for QTL analysis with model 6 at a walking speed of 1 cM (number of control markers = 5; window size = 10 cM). For each trait, the threshold for significant QTLs (P < 0.05) was calculated by a 1000 permutation test. A threshold of 2.5 was used to declare the presence of a QTL. According to Larraza et al. (2002), QTLs were named as Q(m/d)grx, where m is the monokaryotic state, d is the dikaryotic state, gr is the growth rate, and x is a consecutive QTL number. QTLs detected in different media or populations were assumed to be the same when their confidence intervals (CIs) overlapped and the additive values had the same sign (Foulongne-Oriol et al. 2012).

Results

Marker development

A total of 716 polymorphic markers (including MAT-A and MAT-B) were detected in the monokaryotic population. For SSR, 25 out of 56 primer pairs produced a total of 225 clear polymorphic bands. The number of DNA bands for each SSR primer pair ranged from four to 17, with an average of nine. For TRAP, 474 polymorphic markers were obtained from 43 primer pairs, and three to 20 markers were amplified by each primer pair, with an average of 11. Fifteen out of 25 InDel markers were confirmed to be polymorphic in the monokaryotic population.

Linkage analysis and mapping

In the segregation analysis of the 716 polymorphic markers in the monokaryotic population, 134 markers (37 SSR markers and 97 TRAP markers) (18.7 %) showed highly distorted segregation ratios (P < 0.01) and 27 markers (10 SSR markers and 17 TRAP markers) (3.8 %) showed significant segregation distortion (0.01 < P < 0.05). The 134 markers that displayed high distortion were excluded in the downstream linkage analysis, due to the failure to group all the markers into different LGs in a preliminary analysis if they were included. Among the remaining 582 markers, 32 were unable to be grouped into any LG and another 26 markers were identical to others (similarity value = 1.000). Thus, these 58 markers were also excluded from the 582 markers in the final linkage map. Finally, a total of 524 markers (172 SSR markers, 343 TRAP markers, 7 InDel markers, and the MAT-A and MAT-B loci) were mapped into 13 LGs, covering a total length of 1006.1 cM, with an average distance of 2.0 cM between markers (Table 1, Fig 1). The number of markers per group varied from six to 110, with an average of 40. The LG length ranged from 21.6 cM to 134.2 cM, with an average of 77.4 cM. The average marker spacing per LG ranged from 1.0 cM to 4.6 cM. The largest interval between two adjacent markers was found to be 21.6 cM on LG13, and no interval larger than 20 cM was found on the other LGs. A total of 25 (4.8 %) skewed markers (0.01 < P < 0.05) were distributed on nine different LGs, ranging from zero to six for each group. Three segregation distortion regions, defined as regions with three or more closely linked markers exhibiting significant segregation distortion (Paillard et al. 2003), were found on LG3, LG6 and LG7. MAT-A and MAT-B were mapped, respectively, on LG4 and LG11.

Based on the current genetic linkage map, the estimated genome length (Le) of L. edodes was found to be 1066.2 cM (an average between 1058.1 cM as calculated by method one and 1074.2 cM by method two; refer to Materials and Methods for details). Based on this value, the theoretical map coverage was roughly estimated to be nearly 100 % (located within 10 cM of one marker), 99.3 % (located within 5 cM of one marker) and 86.0 % (located within 2 cM of one marker) of the genome. Two-tailed cumulative Poisson distribution test revealed a disproportionate distribution of markers across the 13 LGs (Table 1). Compared to the estimated values, LG1 and LG9 had significantly more markers, while LG8 and LG11 had fewer. Markers showing a significant deviation from the uniform distribution were detected in LG2, LG4, LG9, and LG10 (Table 1).

QTL mapping for vegetative mycelium growth

The estimated growth rates (averages of three repetitions) of different populations of L. edodes and on different media were displayed in Table 1. Compared to the monokaryons, the dikaryons grew faster and had smaller coefficients of variation. One-way ANOVA indicated a significant effect of genotype on vegetative mycelium growth rate in the three populations and media (P < 0.01). Significant effects of tester strains (P < 0.01) and media (P < 0.01) on dikaryotic growth rates were also revealed by two-way ANOVA. Moreover, paired sample t-test suggested significant effects of tester strains on dikaryotic growth rate on the SD (P < 0.01) medium. Correlation analyses between mycelium growth rates in different populations and media were performed using the Pearson procedure (Table 1). In the LQ-15 population, positive
correlations between dikaryotic growth rates on all the three media were revealed. Dikaryotic growth rates on MYG and CYM showed highly positive correlations ($r = 0.646$, $p < 0.01$), while the correlations of MYG and CYM with SD were relatively low (MYG-SD: $r = 0.338$, $p < 0.01$; CYM-SD: $r = 0.275$, $p < 0.01$). Similar results were also found in the LQ-64 population. However, no significant correlations were found between the dikaryotic growth rates of LQ-15 and LQ-64 on CYM and SD media except for the MYG medium. In addition, monokaryotic growth rates correlated positively, but only to a low extent, with dikaryotic growth rates on both MYG ($r = 0.246$, $p < 0.01$) and CYM ($r = 0.171$, $p < 0.05$) in LQ-15.

QTLs detected by the CIM method in different media and populations were listed in Table 4. For monokaryotic growth rate, putative QTLs Qmgr1 and Qmgr2 were detected, respectively, on LG4 and LG9 of the genetic map. The two QTLs together explained 13.28 % of the phenotypic variation. The lengths of the CI (LOD-1 confidence interval) for Qmgr1 and Qmgr2 were 9.5 cM and 6.7 cM, respectively. Both favorable alleles at the two loci came from the parental strain W1-26. Qmgr1 was mapped in the region from 68.1 cM to 77.6 cM of LG4. It was linked to the InDel marker S278R/F, and was adjacent to MAT-A (70.9 cM on LG4). On the other hand, Qmgr2 was located on LG9 (from 43.0 cM to 49.7 cM) and linked to the SRAP marker ME1-EM4-850.

For dikaryotic growth rate, a total of 13 QTLs were detected in the testcross populations LQ-15 and LQ-64, explaining 6.17 %–23.71 % of the phenotypic variation. In LQ-15, two QTLs were located on LG4, and three others were mapped on LG6. The lengths of CI for these five QTLs ranged from 1.1 cM to 6.6 cM. Among these QTLs for dikaryotic growth rate, Qdgr2 was detected across all three media, with high percentages of phenotypic variation explained (PVE). Qdgr4 and Qdgr5 were found in both the MYG and CYM media. Qdgr1, Qdgr2 and Qdgr3 showed negative additive effects, while Qdgr4 and Qdgr5 showed positive additive effects. Qdgr1 was most likely located in a 6.3 cM region (from 64.3 cM to 70.6 cM) on LG4, adjacent to MAT-A. The CI of Qdgr2 partially overlapped with that of Qmgr1 as detected in the monokaryotic population. Both QTLs showed negative additive effects with favorable alleles from W1-26. In LQ-64, a total of eight putative dikaryotic mycelium growth rate-related QTLs were identified, contributing 6.17 %–11.15 % of the phenotypic variation. Qdgr6 and Qdgr7 were located on LG13 and showed negative additive effects. Qdgr8, Qdgr9 and Qdgr11 were scattered on LG1, LG4 and LG2, respectively. Moreover, the CI of Qdgr9 partially overlapped with that of Qdgr2 as detected in LQ-15. Qdgr10, Qdgr12 and Qdgr13 were located on LG7 (Table 4, Fig 1).

**Discussion**

**Molecular markers used in this study**

Efficient and user-friendly molecular markers are highly valuable tools for genotyping of mapping populations. In this study, SRAP and TRAP, two rapid and powerful PCR-based marker techniques, were used to develop markers for genotyping. Compared to anonymous markers, these gene-targeted markers provide ample opportunities to integrate gene information and phenotypic trait variation, and are important for QTL mapping (Shokeen et al. 2011). SRAP and TRAP target potentially functional genes, and may provide an additional avenue to further genetic studies involved in marker–trait association (Li & Quiros 2001; Hu & Vick 2003; Yu et al. 2007). Unlike AFLP, these two techniques require no extensive pre-PCR processing of templates (Liu et al. 2005). They are also highly efficient in generating a large number of markers (Liu et al. 2005; Gao et al. 2007), which is also the case in the current study on *L. edodes*. On average,
nine SRAP and 11 TRAP markers were produced per primer pair. These are comparable to an average of 8.5 AFLP markers reported in a previous study (Terashima et al. 2006). The application of SRAP and TRAP markers greatly improved both the marker density and the total coverage of the genetic map in *L. edodes*.

Apart from SRAP and TRAP markers, InDel markers were also used in this study. These markers were derived from the *L. edodes* draft genome sequence (unpublished data in Prof. Kwan’s lab), which was not available in previous studies. Compared with AFLP, these sequence-based markers could facilitate the integration of genetic map and genome sequence. However, only seven InDel markers were available in the current genetic map, making it difficult to establish a correlation between the genetic map and the genome sequence. More InDel markers are thus desired to improve the present genetic map. To our knowledge, this is the first report of using SRAP, TRAP and InDel markers for genetic linkage mapping in edible mushrooms.

In the monokaryotic population, 22.5 % of the markers found to be skewed. This is similar to a value (20.7 %) reported previously (Terashima et al. 2002). Several hypotheses have been proposed to explain distorted segregation, such as biased selection of SSIs, expression of lethal factors and unbalancing selection of mating-types (Larraza et al. 2000; Fouloungne-Oriol et al. 2010). The mapped skewed loci appeared to locate together as segregation distortion regions in the genetic map, as previously reported in *L. edodes* (Terashima et al. 2002), *P. pulmonarius* (Okuda et al. 2009) and *P. eryngii* (Okuda et al. 2012). The cluster distribution of skewed loci seemed to indicate that the segregation distortion of markers is most likely caused by genetic factors rather than statistical bias (Li et al. 2010).

**Features of the current genetic map**

A genetic map with the highest marker density in *L. edodes* to date was constructed here using SRAP, TRAP and InDel molecular markers. Compared to previous studies (Table 5), the current study involved the largest mapping population (146 SSIs), 1006.1 cM, with an average marker distance of 2.0 cM. In terms of total length, the current map was close to that constructed by tetrad analysis (908.8 cM) (Miyazaki et al. 2008), shorter than that of the AFLP-based map (1398.4 cM) (Terashima et al. 2006), and longer than those of the other two maps (Kwan & Xu 2002; Au et al. 2013).

Thirteen LGs were revealed in this study. The LGs outnumbered the chromosomes of *L. edodes* as revealed using contour-clamped homogeneous electric field (CHEF) gel electrophoresis, in which only eight chromosomes were found in a ~ 33 Mb genome (Arima & Morinaga 1993). Recently, the genome of *L. edodes* has been sequenced and the total length of the assembly was estimated to be 40.2 Mb (Kwan et al. 2012). Therefore, there may be more than eight chromosomes in *L. edodes*. Indeed, the number of chromosomes in *L. edodes* has remained unclear (Terashima et al. 2002). As demonstrated in *A. bisporus* and *Flammulina velutipes*, the assignment of DNA probes designed from genetic linkage maps to CHEF-separated chromosomes may be a more effective karyotyping approach (Sonnenberg et al. 1996; Tanesaka et al. 2012). Additional experiments on hybridization of genetically mapped markers to CHEF blots are thus needed to reveal the actual number of chromosomes in *L. edodes*. In this study, a larger LG number could be attributed to an absence of suitable markers to link LGs belonging to the same chromosome, as well as high LOD values (5.0–8.0 in some cases) used for grouping in linkage mapping. The number of markers mapped in previous genetic maps of *L. edodes* varied from 69 to 289 (Table 5) whereas 524 markers were mapped in the current map. The average marker distance reported here (2.0 cM) is shorter than those in any previously reported genetic maps (Table 5). There was only one gap (>20 cM) reported in the current map.

The MAT-A locus was located on a relatively large LG in the current map. The same finding has been reported previously (Terashima et al. 2006; Miyazaki et al. 2008; Au et al. 2013). MAT-A and the InDel marker S278/F, derived from scaffold S278 of the shiitake draft genome (unpublished data in Prof. Kwan’s lab), were located on the same LG, which was in congruence with previous findings (Au et al. 2013). Both the InDel marker DTF1R/F derived from scaffold S214 (Supplemental Table S3) and MAT-B also belonged to the same LG as previously reported (Au et al. 2013). LG4 and LG11 in our map corresponded respectively to LG2 and LG11 in the map constructed by Au et al. (2013).
Fig 1 – (continued).
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The physical distance per unit of recombination for L. edodes was roughly estimated to be 40.0 kbp/cM in our map, which was close to that of P. ostreatus (35.1 kbp/cM) (Larraya et al. 2000) and A. bisporus (33.1 kbp/cM) (Foulongne-Oriol et al. 2010).

**QTLs controlling the vegetative mycelium growth rate**

For fundamental and applied interest, the dissection of genetic architecture of mycelium growth has been carried out in edible mushrooms using QTL mapping or other methodologies (Larraya et al. 2001, 2002; Foulongne-Oriol 2012; Sivolapova et al. 2012). In this study, we used three different segregation populations to uncover the loci regulating and controlling the vegetative mycelium growth rate of L. edodes. Two QTLs accounting for the monokaryotic mycelium growth rate were detected using SSIs, whereas 13 dikaryotic mycelium growth rate-related QTLs were identified based on two testcross dikaryotic populations across three different media. Notably, several vegetative growth rate-related QTLs in L. edodes uncovered here were clustered on LG4 (Qmgr1, Qdgr1, Qdgr2, and Qdgr9) and LG6 (Qdgr3, Qdgr4 and Qdgr5). Among them, the robust QTL Qdgr2 was found in three media in LQ-15, contributing 8.07 %–23.71 % of the phenotypic variation. These results suggested the predominant role of a few principal genomic regions in regulating the vegetative mycelium growth rate.

Qmgr1, Qdgr2 and Qdgr9 were all linked with the InDel marker S278R/F, which was 0.3 cM away from MAT-A. Qmgr1 and Qdgr2 probably corresponded to the same gene since they were mapped on consistent locations with the same negative additive effect, while Qdgr9 was probably on a different locus with a different sign in the additive value. The linkage between MAT-A and Qmgr1, Qdgr1, Qdgr2, and Qdgr9 revealed an association of vegetative mycelium growth with sexual recognition in L. edodes. Indeed, similar associations have been observed in P. ostreatus (Larraya et al. 2001; Sivolapova et al. 2012) and Amylostereum areolatum (van der Nest et al. 2009), in which the MAT-A locus was positioned in the same region as the putative significant QTLs associated with mycelium growth rate. A possible explanation for the association is that the recognition loci are subject to evolutionary forces (balancing selection and suppressed recombination) that are markedly different from those acting on the rest of the genome (van der Nest et al. 2009). It is likely that mycelium growth is influenced by balancing selection acting on its recognition loci, or the genomic regions flanking these loci (van der Nest et al. 2009).

QTLs for dikaryotic mycelium growth rates detected in LQ-15 and LQ-64 showed no consistency in location, indicating strong effects of the two compatible tester strains on dikaryotic mycelium growth rate. The localization of QTLs was different on different media in the same population, implying a considerable effect of the substrate on phenotypic variation. SD is a complex medium used to produce shiitake mushroom, therefore QTLs detected in it would provide more instructive clues for breeding schemes.

In our study, some TRAP markers, generated from several different on different media in the same population, implying a considerable effect of the substrate on phenotypic variation. SD is a complex medium used to produce shiitake mushroom, therefore QTLs detected in it would provide more instructive clues for breeding schemes.
controlling the vegetative mycelium growth rate (Table 4, Fig 1). These genes included cell wall degradation-related enzyme-encoding genes (mlg1), putative fungal-specific transcription factor (ftf2) and the glyceraldehyde-3-phosphate dehydrogenase gene (gpd). The mlg1 gene encodes a glucanase of the GH16 family (Sakamoto et al. 2009), which could break down glucans. The TRAP marker, mlg1-2-420, was found to be linked to Qdgr12, suggesting an involvement of mlg1 in carbohydrate degradation of L. edodes in the mixed SD medium. In L. edodes, gpd is expressed constitutively and strongly during each stage of fruiting-body development (Hirano et al. 1999). This gene encodes a key enzyme in both glycolysis and gluconeogenesis, and may serve to supply energy and substrates for mycelium growth. The fungal-specific transcription factor ftf2 encodes proteins that contain a Zn(2)-C6 fungal-type DNA-binding domain in the N-terminal, as well as a fungal-specific transcription factor domain at the center (Sakamoto et al. 2009). In Candida albicans, some transcription factors containing the Zn(2)-C6 motif were found to be involved in filamentous growth (MacPherson et al. 2006). TRAP markers could target potentially functional genes and may provide additional clues for the conversion of mapped QTLs to candidate genes.

**Conclusions**

Molecular breeding is a powerful tool for genetic improvement of crops and animals. However, there is still a long way to utilize this method in genetic improvement of edible mushrooms due to the deficiency of genetic research on their important economic and agronomic traits. In the present study, a high-quality genetic map of L. edodes was constructed, and a total
of 15 putative QTLs for the vegetative mycelium growth rate were detected in the subsequent QTL mapping. A QTL hotspot region on LG4 of the current genetic map, which was found to be associated with both the monokaryotic and dikaryotic mycelium growth rates, was reported here. The present study demonstrates the potential of using the current linkage map to identify QTLs for traits that are more difficult to measure in L. edodes, such as yield and yield-related traits. In summary, this study lays a solid foundation for establishing efficient genetic and breeding programs in L. edodes. With the completion of genome sequencing of L. edodes (Kwan et al. 2012), more and more sequence tag site markers, such as simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers, are expected to be developed for studies on genetic map construction and QTL mapping of L. edodes.

Disclosure statement
None of the authors has any conflict of interest to disclose.

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Appendix A. Supplementary data
Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funbio.2014.01.001

REFERENCES


