Genetic analysis of segregation distortion of mating-type factors in *Lentinula edodes* *

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Abstract Analyses of the mating-type ratio of protoplast monokaryons to spores in 28 strains of *Lentinula edodes* were performed through statistical methods. The results showed that the segregation distortion phenomenon commonly exists in both types of *Lentinula edodes*. 17 out of the 28 samples did not display the expected 1∶1 ratio of protoplast monokaryons; and among them 8 fruiting strains distorted 1∶1∶1∶1 segregation of spores. The degree of distortion in all tested wild strains was significantly lower than that of cultured strains. In fruiting test of positive-negative parent, predominant spores were those with the mating type identical to dikaryotic parent, indicating that the predominance of nuclei mainly depends on the mating type of dikaryotic parent. Data from di-mon mating test verified that monokaryons with acceptor nuclear type are present exclusively in a large amount after dedikaryotization, suggesting that the specificities among B factors and some other unknown factors in cytoplasm are probably responsible for the phenomenon of skewed segregation.

Keywords: *Lentinula edodes*, mating-type factors, segregation distortion, cytoplasm.

Lentinula edodes (Berk.) Pegler (Shiitake or Xianggu mushroom) is the second in total production of cultivated mushroom in the world. China is a leading producer and exporter of *Lentinula edodes*, accounting for 80% of the world total production[1]. Shiitake is a heterothallic homobasidiomycete whose mating is controlled by a bifactorial tetrapolar genetic system. Two mating factors A and B, sited respectively on two different chromosomes, control different steps of hyphal fusion, nuclear migration and nuclear sorting during the onset and progress of the dikaryotic growth[2]. Theoretically, if two mating types are isolated from a dikaryon and four mating types originated from the products of meiosis, they should be evenly distributed.

In recent years, the phenomenon of segregation distortion of mating type factors has been discovered in a variety of fungi, such as *Phytophthora infestans* (Mont.) De Bary[3], *Schizophyllum commune* (L.) Fr.[4], *Flammulina velutipes* (Fr.) Sing.[5] and *Pleurotus ostreatus* (Tacg. ex Fr.) Quel.[6]. In *Lentinula edodes*, segregation distortion has also been observed not only in protoplast monokaryons but also in spores since the 1990s[7—9]. But what is the meaning of unbalanced segregation? Does difference in the degree of segregation distortion between wild strains and cultivated strains exist? What is the genetic basis of this specific phenomenon? These questions remain unanswered. In this study, we analyzed the mating-type ratio of protoplast monokaryons to spores in 28 strains of *Lentinula edodes* to explain the meanings of this segregation distortion phenomenon.

1 Materials and methods

1.1 Strains and culture media

The strains used in this study are listed in Table 1. The media MYG (malt extract, glucose and yeast extract), PDY (potato extract, dextrose and yeast extract), CYM (standard complete medium) and sawdust mixture were prepared as described in Ref. [9].

1.2 Cultivation of fruit bodies

Polythene bags were filled with a sawdust mixture, sterilized by autoclaving and inoculated with *Lentinula edodes* mycelia. The mycelia were grown in the sawdust substrates for 70 days in the dark at 25 ℃, after which the cultures were subjected to illumination at 18 ℃. Fruit bodies were harvested after

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about 1 month.

Table 1. Strains of *Lentinula edodes*

Strains	Original sources	Strains	Original sources
IB01[a)]	IBHAS[b)]	SHX002	Shanxi
IB02	IBHAS	SHX020	Shanxi
IB03	IBHAS	SHX021	Shanxi
IB08	IBHAS	SHX039	Shanxi
IB09	IBHAS	SHX041	Shanxi
IB10	IBHAS	HUB007	Hubei
IB14	IBHAS	HUB021	Hubei
IB15	IBHAS	HUB028	Hubei
IB19	IBHAS	HUB049	Hubei
IB29	IBHAS	HUB087	Hubei
IB31	IBHAS	YUN013	Yuannan
WL-1	MSCHAU[c)]	YUN102	Yuannan
GAN054	Gansu	YUN039	Yuannan
GAN057	Gansu	YUN109	Yuannan

a) IB and WL are cultivated strains, the others are wild type strains; b) IBHAS, Institute of Biology, Henan Academy of Science; c) MSCHAU, The Mushroom Spawn Center of Huazhong Agricultural University

1.3 Spores isolation

Spores were collected using the method of discharging spores from the basidiocarp of the respective dikaryotic parents. Single spores were obtained by the dilution method. Spore cultures of each strain were isolated by picking up single spores individually under a dissecting microscope and transferred to CYM medium, and a putative homokaryotic mycelium was confirmed by the lack of clamp connections.

1.4 Dedikaryotization and analysis of mating types

Monokaryotic components of dikaryons were isolated via protoplast manipulations according to Lin's method[9]. The mating types of protoplast monokaryons and spores were determined as described in Refs. [9] and [10]. Ratio of protoplast monokaryons was subject to statistical analysis using the formula $x^2 = \frac{(|A-a|-1)^2}{n}$, where A and a are the experimental observation values, and $n = A + a$. Analysis of the ratios of four mating types among spore monkaryons was performed using statistical formula $x^2 = \sum_1^k \frac{(O-E)^2}{E}$, where O and E represent the experimental observation value and expected value respectively.

1.5 Progeny analysis of di-mon mate

The experimental procedures for progeny analysis of di-mon mate were all based upon the methods presented in Refs. [11] and [12].

2 Results

2.1 Mating type ratio of protoplast monokaryons and spores

Table 2 summarizes the results of mating tests. In all 28 tested strains two mating types were recovered from dikaryotic strain except GAN057, which was determined as the only one mating type among 56 randomly selected protoplast monokaryons, the ratio of two mating types varied and did not show a 1∶1 ratio in most cases. Among the 28 tested strains, 17 strains, including 8 out of 12 cultivated strains and 9 out of 17 wild strains, displayed unbalanced distribution of two mating types.

Table 2. Ratio between two types of protoplast monokaryons and among four types of spores

Strains	Ratio of protoplast monokaryons	χ^{2} [a)]	Ratio of spore monokaryons	χ^{2} [b)]
IB01	36∶24	2.02	21∶19∶13∶10	5.00
IB02	46∶34	1.15	20∶20∶17∶3	13.20
IB03	60∶6	42.56	23∶18∶13∶8	7.83
IB08	38∶26	1.89	18∶21∶13∶6	6.53
IB09	36∶18	5.35	21∶19∶21∶12	3.00
IB10	47∶19	11.05	25∶23∶17∶11	6.32
IB14	46∶15	14.75	56∶24∶12∶8	56.60
IB15	47∶11	21.12	21∶19∶15∶10	4.91
IB19	51∶29	5.51	40∶24∶0∶0	72.00
IB29	50∶14	19.14	12∶45∶6∶2	70.94
IB31	48∶18	12.74	25∶19∶13∶8	10.02
WL-1	34∶21	2.62	37∶44∶42∶30	3.05
SHX021	79∶15	42.22	20∶18∶20∶12	2.46
SHX002	42∶33	0.98	20∶16∶19∶15	0.97
HUB021	52∶46	0.26	28∶74∶61∶39	26.53
GAN054	39∶15	9.79	——	
SHX020	54∶7	34.69	——	
SHX039	44∶36	0.61	——	
HUB007	53∶17	17.50	——	
HUB028	37∶15	8.48	——	
HUB049	38∶22	3.75	——	
HUB087	52∶28	6.61	——	
YUN013	44∶16	12.15	——	
YUN039	42∶23	4.98	——	
YUN102	60∶40	3.61	——	
YUN109	32∶28	0.15	——	
SHX039	44∶36	0.61	——	
HUB007	53∶17	17.50	——	

a) For significance at 5% = 3.84, at 1% = 6.63, the degree of freedom = 1; b) for significance at 5% = 7.81, at 1% = 11.3, the degree of freedom = 3

Moreover, four mating types in 17 fruiting

strains were identified except IB19, which was the only one with two parental nuclear types with four mating types among 64 spore monokaryons. The observed ratio of four mating types varied. In 8 out of the 17 tested strains, including 6 cultivated strains and 2 wild strains, we did not find a 1∶1∶1∶1 ratio and we divided spores into two groups based on their mating types: parental type and non-parental type. It was found that the ratio between the two groups skewed 1∶1, in which the spores with parental nuclear type (AxBx+AyBy) outnumbered those with non-parental type (AxBy+AyBx) in most strains (Data not shown).

2.2 Ratio of spores with parental mating type to those with non-parental type

To test if parental type spores always outnumbered non-parental (also called recombinant) type spores in most strains as described above, three hybrid dikaryotic strains RM03, RM10 and RM14 were generated via mating the two non-parental mating type spores derived respectively from strains IB03, IB10 and IB14 to analyze the ratio of progeny spores.

From Table 3, it can be clearly observed that no matter whether dikaryotic mycelia with mating type AxBx+AyBy (in strains IB03, IB10 and IB14) or those generated by mating recombinant spores AxBy with AyBx (in strains RM03, RM10 and RM14) served as parental strain, predominant spores were those with the mating type identical to that of the dikaryotic parent, indicating that the predominance of nuclei mainly depends on the mating type of dikaryotic parent strains. This result further confirmed that spores with parental type always outnumbered non-parental type spores.

Table 3. Data of positive-negatative parental fruiting

Strains	Origins	Parental dikaryons	(AxBx+AyBy)∶(AxBy+AyBx)	χ^{2} a)
IB03	IB03	AxBx+AyBy	41∶21	5.82
Rm03	IB03s9b)× IB03s18	AxBy+AyBx	40∶70	7.65
IB10	IB10	AxBx+AyBy	48∶28	4.75
RM10	IB10s19× IB10s47	AxBy+AyBx	80∶114	5.61
IB14	IB14	AxBx+AyBy	80∶20	34.81
RM14	IB14s28× IB14s38	AxBy+AyBx	32∶64	10.01

a) For significance at 5%=3.84, at 1%=6.63, the degree of freedom = 1; b) F1 progeny sporulated monokaryon of strain IB03, No.9

2.3 Dikaryon-monokaryon (di-mon) mating analysis

From Table 4, it can be observed that only one of the two component nuclei of dikaryon can migrate into the monokaryon, and the difference in transferred nucleus (called "pre-entrance nucleus") is determined by its ability of combining with acceptor nucleus and cytoplasm environment of monokaryon crossed with dikaryon. For example, when a dikaryon [A_1B_1+A_2B_2] was crossed with monokaryon A_3B_3 or A_4B_4, the pre-entrance nuclei were the same A_1B_1, whereas when dikaryon [A_3B_3+A_4B_4] crossed with monokaryon A_1B_1 or A_2B_2, the pre-entrance nuclei were respectively A_4B_4 with A_1B_1 as an acceptor nucleus and A_3B_3 in the case of A_2B_2 as an acceptor nucleus. Similarly, when a dikaryon [A_5B_5+A_6B_6] was crossed respectively with monokaryon A_7B_7 and A_8B_8, the pre-entrance nuclei were the same A_5B_5, but when dikaryon [A_7B_7+A_8B_8] crossed respectively with monokaryon A_5B_5 and A_6B_6, the pre-entrance nuclei were respectively A_7B_7 and A_8B_8. Moreover, the derived dikaryons of crosses [A_1B_1 + A_2B_2] × A_4B_4 and [A_3B_3 + A_4B_4] × A_1B_1 were the same, namely, [A_1B_1 + A_4B_4]. The result indicated that during dikaryotization, the combined ability of A_1B_1 with A_4B_4 is higher than that of A_1B_1 with A_3B_3 and A_2B_2 with A_3B_3 and A_4B_4. Similarly, the variation of combined ability between different mating-type nuclei appeared in the crosses of [A_5B_5+A_6B_6] × A_7B_7 and [A_7B_7+A_8B_8] × A_5B_5, the pairing of A_5B_5 and A_7B_7 has a priority to that of A_5B_5 with A_8B_8, A_6B_6 with A_7B_7 and A_6B_6 with A_8B_8.

From Table 4, we may find that protoplast monokaryons with the matingtype of acceptor nucleus outnumber exclusively those with the matingtype of pre-entrance nucleus in all 8 derived dikaryons after dedikaryotization. Worthily mentioned, there are two derived dikaryons with the same mating-type [A_1B_1 +A_4B_4], one is the product of cross [A_1B_1+A_2B_2] × A_4B_4 mating and the other originated from cross [A_3B_3+A_4B_4] × A_1B_1. The ratio of two mating types

(A_1B_1 : A_4B_4) among monokaryons derived from protoplast manipulation is 62 : 13 in the first case and 21 : 51 in the latter. Similarly, this phenomenon occur in derived dikaryon [A_5B_5 + A_7B_7], one is the product of cross [A_5B_5 + A_6B_6] × A_7B_7 and the other is that of cross [A_7B_7 + A_8B_8] × A_5B_5, the ratio of A_5B_5 and A_7B_7 is 32 : 58 and 60 : 27 respectively. The result suggests that the unbalanced ratio is probably due to some factors in cytoplasm that influence survival of nuclei recovery from dedikaryotization.

Table 4. The results and analysis of di-mon hybrids of *Lentinula edodes*

Strains	Orgins	Cross combinations	Matingtype of derived dikaryon	Recipient/donor	χ^{2} a)
1	IB01× IB15s66	(A1B1+A2B2)×A3B3	A3B3+ A1B1	54 : 18	17.01
2	IB01× IB15s64	(A1B1+A2B2)×A4B4	A4B4+ A1B1	51 : 21	11.68
3	IB15× IB1s41	(A3B3+A4B4)×A1B1	A1B1+ A4B4	62 : 13	30.72
4	IB15× IB1s37	(A3B3+A4B4)×A2B2	A2B2+ A3B3	49 : 26	6.45
5	HUB007× SHX021s2	(A5B5+A6B6)×A7B7	A7B7+ A5B5	58 : 32	7.81
6	HUB007× SHX021s31	(A5B5+A6B6)×A8B8	A8B8+ A5B5	73 : 22	27.78
7	SHX021× HUB007s2	(A7B7+A8B8)×A5B5	A5B5+ A7B7	60 : 27	11.77
8	SHX021× HUB00s13	(A7B7+A8B8)×A6B6	A6B6+ A8B8	64 : 30	11.59

a) For significance at 5% = 3.84, at 1% = 6.63, the degree of freedom = 1

4 Discussion

Segregation distortion phenomenon is very common in nature and increasingly recognized as a potentially powerful evolutionary force[12]. The several possible mechanisms are: (1) the existence of balance lethal loci or recessive pure lethal alleles linked with mating type locus[13]; (2) the different origin of materials used in different research[14]; (3) slight influence of cytoplasmic genome of single parent[15]; (4) the existence of structural rearrangement, deletion, insertion and mutation in the course of inter-chromosome exchange[16].

Some explanations to segregation distortion of mating type factors in a variety of fungi were proposed. In *Flammulina velutipes*, the asymmetric ratio of the two protoplast monokaryons was possibly related to the existence of fertile-inhibitors linked with A and B as well as the selection of cytoplasm to survival nucleus[4]. The skewed ratios of one type of nucleus to the other in *Schizophyllum commune* attributed to the difference of B-mating-type alleles[5], which influences nuclear survival. Those alleles can be put in a hierarchical order with respect to this function. Mutation to loss or impairment of B function results in a shift in the hierarchical order related to the progenitor alleles. In *Phytophthora infestans*, it was identified that the mating type locus displaying non-Medelian segregation is the result of existence of balance lethal loci near the mating type locus[3]. By his work, Judelson explained the molecular mechanism of segregation distortion of mating type through cloning, sequencing and mapping to the balance lethal loci[17].

In this study, we investigated the distribution of mating type between protoplast monokaryons and among spores and found that segregation distortion phenomenon commonly exists in *Lentinula edodes*. To some extent, the degree of skewed ratios of mating type not only between protoplast monokaryons but among spore monokaryons in tested wild strains is lower than that of cultivated strains tested.

The development of haploid spore is the result of natural meiosis during sexual reproduction with no artificial factors involved. Why does the significantly unbalanced segregation among spore monokaryons occur? In the course of fruit-body formation, no matter whether AxBx cross-mated with AyBy or AxBy with AyBx, following plasmogamy and karyogamy, the constitute of diploid nucleus is the same: AxAyBxBy. According to the rule of Medenlian segregation, the ratio of four nuclear types of spores should present theoretically 1 : 1 : 1 : 1 after meiosis. The existence of a tendency that the predominant spores among spores are always those with parental nuclear types may be correlated with the specificity of different B-mating-type factors associated with A factors, or their products during fruit-body formation.

Compatibility in *Lentinula edodes* requires heteroallelism at both of two complex genetic regions, the A and B mating factors. Heteroallelism of the B factor allows extensive nuclear migration of one nuclear type through the hyphae of a monokaryotic mate. Heteroallelism of the A factor allows other aspects of

the mating reaction needed for the establishment of a balanced, clamped dikaryon. The data in this study showed that specificity of B factor is correlated with the sort of migration nucleus, the higher the specificity, the faster the rate of migration of donor nucleus. Monokaryons with accepter mating-type outnumbered exclusively those with mating-type of pre-entrance, probably due to some factors existing in cytoplasm, which influences survival of nuclei recovery from dedikaryotization. But we do not know which factor is responsible for this special genetic phenomenon at present time.

However, to provide further information concerning genetic factors that influence segregation of mating type factors in *Lentinula edodes*, many other factors should be considered, such as the existence of fertile- inhibitors linked with mating-type factors of A or B, balance lethal loci near the mating-type locus, intra-locus recombination between A and B mating-type factors, recessive pure lethal alleles linked with mating types, unknown recessive factors near the mating type and the other unknown factors.

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