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Mapping of Genomic Regions (Quantitative Trait Loci) Controlling Production and Quality in Industrial Cultures of the Edible Basidiomycete *Pleurotus ostreatus*

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Industrial production of the edible basidiomycete *Pleurotus ostreatus* (oyster mushroom) is based on a solid fermentation process in which a limited number of selected strains are used. Optimization of industrial mushroom production depends on improving the culture process and breeding new strains with higher yields and productivities. Traditionally, fungal breeding has been carried out by an empirical trial and error process. In this study, we used a different approach by mapping quantitative trait loci (QTLs) controlling culture production and quality within the framework of the genetic linkage map of *P. ostreatus*. Ten production traits and four quality traits were studied and mapped. The production QTLs identified explain nearly one-half of the production variation. More interestingly, a single QTL mapping to the highly polymorphic chromosome VII appears to be involved in control of all the productivity traits studied. Quality QTLs appear to be scattered across the genome and to have less effect on the variation of the corresponding traits. Moreover, some of the new hybrid strains constructed in the course of our experiments had production or quality values higher than those of the parents or other commercial strains. This approach opens the possibility of marker-assisted selection and breeding of new industrial strains of this fungus.

The edible mushroom production industry is more important every year for several reasons: mushrooms are an efficient low-fat protein source, they may be cultivated on a wide variety of substrates, and they have many industrial and medical applications. The oyster mushroom (*Pleurotus ostreatus* Jacq. ex Fr Kummer) is the second largest edible mushroom crop behind the white button mushroom, *Agaricus bisporus*, and it accounts for nearly one-quarter of the total worldwide mushroom production (4, 5). This fungus is also a source of enzymes and other products with industrial and medical applications (6, 11, 12, 23), and it can be used as a decontaminating agent (3) and as an organic fertilizer (1).

Industrial production of *P. ostreatus* is based on a two-step solid fermentation process. In the first step, the mycelium colonizes a wheat (or cereal) straw-based substrate under light- and oxygen-limited conditions at an incubation temperature of 24°C. This process takes between 1 and 2 weeks before the mycelium reaches the substrate surface. At this time, the incubation conditions are changed (12-h photoperiod, forced ventilation) to induce successive flushes of mushroom fruiting. The fruiting temperature depends on the strain and varies between 15°C (*P. ostreatus* var. *ostreatus*) and 21°C (*P. ostreatus* var. *florida*).

Optimization of *P. ostreatus* industrial fermentation is based primarily on process improvement and strain improvement. Whereas much work has been done on process improvement (27), few systematic studies of genetic breeding of *P. ostreatus* strains have been reported. This is also true for other industrially cultured mushrooms.

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Understanding mushroom breeding systems is a major landmark when commercial breeding programs are being established. *P. ostreatus* production is dependent on the life cycle, which alternates between monokaryotic (haploid nucleus) and dikaryotic (dihaploid nucleus) phases (10). Two compatible monokaryotic hyphae are able to fuse and give rise to a dikaryotic mycelium in which the two parental nuclei remain independent (dikaryon, heterokaryon) throughout vegetative growth and fruiting body development. True diploidy occurs only in the basidia, where karyogamy takes place immediately before the meiotic division that produces four uninucleate basidiospores.

Mushroom breeding and selection (development, evaluation, and maintenance of new genotypes) have progressed at different rates for different species. In general, little information is available regarding the genetic control of traits such as yield or quality because of the difficulty in assessing the traits (which often depend on environmental and substrate conditions and likely are inherited in a complex manner) in small-scale tests and the lack of suitable genetic tools that allow integration of molecular techniques with small-scale industrial cultivation assays. Consequently, breeding high-yielding strains has traditionally been accomplished by trial and error, and large numbers of hybrids, obtained by pairing monosporic cultures, need to be cultivated to evaluate the production characteristics (7).

In the last few years, our group has developed some genetic tools for *P. ostreatus* (including molecular markers linked to the mating genes [16, 18] and karyotype and genetic linkage maps [19, 20]) that have allowed genetic analysis of quantitative traits (quantitative trait loci [QTLs]), such as mycelium growth rate (17). In this paper we describe mapping of QTLs that control some important industrial production and quality traits, such as yield, precocity, cap color, tolerance to high

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TABLE 1. Production and quality traits

Trait[a]	Definition	Designation[b]
Precocity[c]	Time (in days), from the start of incubation under fruiting conditions until harvest of the first fruiting body bunch	P_{21}, P_{15}
Effect of the temperature on precocity	Difference between P_{15} and P_{21}	$P_{15\times21}$
Yield[c]	Ratio (expressed as a percentage) of the weight of the fruiting bodies harvested during the cropping time to the weight of the substrate (2 kg)	Y_{21}, Y_{15}
Effect of temperature on yield	Difference between FFY_{15} and FFY_{21}	$Y_{15\times21}$
First-flush yield[c]	Ratio (expressed as a percentage) between the weight of the fruiting bodies harvested during the first fruiting flush and the weight of the substrate (2 kg)	FFY_{21}, FFY_{15}
Fruiting body number[d]	Number of fruiting bodies collected during the cropping time	NUM
Clean fruiting body weight[d]	Fruiting body average weight	CFW
Stipe weight loss[d]	Percentage of the fruiting body weight lost as a consequence of elimination of the stipes	SWL
Cold-storage weight loss[d]	Percentage of the fruiting weight lost after storage of the pilei for 7 days at 4°C	CWL
Fruiting body fleshiness[e]	Pileus thickness estimated by determining the ratio of pileus weight to surface, assuming the same density for all genotypes (in grams per square centimeter)	FLE
Fruiting body color[e]	Pileus surface color measured by using the tristimulus coordinate system CIELAB scale	COL

[a] The production traits are precocity, yield, first-flush yield, fruiting body number, and clean fruiting body weight. The quality traits are stipe weight loss, cold-storage weight loss, fruiting body fleshness, and fruiting body color.
[b] Most traits were determined at 21°C; the exceptions were precocity, yield, and first-flush yield, which were also determined at 15°C. In the latter cases, the subscript indicates the temperature (in degrees Celsius) at which data were collected.
[c] Calculated by using all of the data obtained during the cropping time.
[d] Calculated by using data from selected harvests.
[e] Calculated by using data for selected fruiting bodies from selected harvests.

temperatures, etc., in *P. ostreatus*. To our knowledge, this is the first report of QTL analysis related to production and quality characteristics in edible mushrooms, and our findings establish a basis for future marker-assisted selection breeding programs in this and other systems.

MATERIALS AND METHODS

Fungal strains and populations. All the *P. ostreatus* strains used in this work have been described previously (17, 18). Two hybrid commercial strains (strains 200 and 201, kindly provided by Sylvan-Somycel S.A., Langeais, France) were used as reference organisms for comparisons of the quality and production values of hybrids. Monokaryotic and dikaryotic cultures were grown in the lab at 24°C in the dark and were stored in petri dishes at 4°C in solid Eger medium (20 g of malt extract per liter, 15 g of agar per liter) (9) and under industrial conditions as described below.

A segregating population consisting of 80 dikaryotic individuals (population D80) was used for mapping QTLs that control production and quality. Population D80 was produced by mating monokaryotic tester strain PC21 with the progeny of 80 monokaryons (population M80) derived from the dikaryotic commercial strain N001 (*P. ostreatus* var. *florida*). Monokaryotic population M80 had been used previously for construction of a genetic linkage map of *P. ostreatus* based on the segregation of 200 molecular markers (20). For mapping of some traits, a larger dikaryotic population (population D130) was used. D130 was constructed by mating 50 new monokaryons derived from dikaryotic strain N001 to the same tester strain, PC21, and adding the new hybrids to population D80 for statistical analysis. Tester strain PC21 is one of the two protoclones (monokaryons carrying only one of the nuclei present in a dikaryotic strain) of wild dikaryotic strain N003 (*P. ostreatus* var. *ostreatus*) and is compatible with all members of progeny populations M80 and M130.

To obtain the dikaryotic populations, inocula consisting of about 16 mm^2 of mycelia of the two mating monokaryons were placed 4 cm apart in the center of a petri dish and incubated at 24°C in the dark until a large contact zone formed. A piece of mycelium was then cut off the overlapping area and subcultured at 24°C in the dark. In order to confirm true dikaryosis, the mycelium of the subculture was examined with a microscope to determine the presence of clamp connections (16, 18).

Experimental design and culture conditions. All the dikaryons produced were tested for fruiting under commercial conditions. A substrate based on pasteurized wheat straw with a moisture content of 70% was used for the production assays. The spawn for each of the 80 or 130 members of the dikaryotic population was grown on boiled and sterilized millet grains. For the fruiting assays, six plastic bags were prepared for each individual by mixing the corresponding spawn with the straw substrate at a proportion of 2% (wt/wt), and then the spawn-containing substrates were packed in plastic bags with holes (2 kg per bag) and incubated at 25°C in the dark until the mycelium spread all over the substrate (approximately 2 weeks). Once the bags were colonized by the mycelium, they were transferred to fruiting conditions (90% environmental, 12 h of light and 12 h of darkness, and ventilation) and incubated at either 15 or 21°C (three bags of each individual per temperature). The fruiting assays were performed with 80 or 130 different dikaryons. Eighty dikaryons were used to map production traits, while in most of cases 130 dikaryons were used to analyze quality traits.

Traits studied and data collection. The production and quality traits studied are described in Table 1. The fruiting experiments were performed simultaneously at both temperatures (15 and 21°C). The cropping period lasted for 1 month after harvest of the most precocious fruiting bodies. Mature fruiting bodies were collected and weighted daily during this period. Some of the traits studied were examined by using all the data obtained during the cropping time; other traits were studied by using data from selected harvests; and still other traits were studied by using data from selected fruiting bodies from selected harvests (Table 1). In the latter cases, three measurements per genotype (one per sack) were obtained for the harvest on a day when production was high.

Fruiting body color was measured by using the tristimulus coordinate system CIELAB scale (Commission Internationale de l'Eclairage 1976, L^*a^*b color system), which expresses any color as three parameters, L (lightness) and a and b (chromaticity coordinates). The L reference value varies from 0 (black) to 100 (white), a ranges from red (positive reading) to green (negative reading), and b varies from yellow (positive reading) to blue (negative reading). Color measurements were made with a Minolta Chromameter (CM500) by using illuminant D65 just after harvest. The measurements analyzed in this work correspond to the average lightness between a central point and a border point of the pileus.

Statistical analyses. The data for each quantitative trait were subjected to normality tests (Kolmogorov-Smirnov), and the effect of the genotype on every trait was determined by one-way analysis of variance by using the SPSS for Windows V8.0.1S software (SPSS Inc., Chicago, Ill.). Correlation coefficients for pairs of characters were determined by using the Pearson procedure (SPSS Inc.).

QTLs were mapped by using the least square interval mapping methods developed for backcross progenies as described by Knapp et al. (13, 14); the value used for each genotype was the average of the values for three repetitions

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TABLE 2. Quantitative trait estimates: means, ranges, coefficients of variation

Trait	Mean[a,b]	Range of variation[b]	Coefficient of variation (%)
Production traits			
P_{21}	7.19	17.67	47.17
P_{15}	11.27	25.67	43.99
$P_{15\times21}$	3.95	17.67	61.83
Y_{21}	19.42	18.70	18.83
Y_{15}	13.36	19.85	29.87
$Y_{15\times21}$	1.89	16.38	151.51
FFY_{21}	11.26	14.62	28.06
FFY_{15}	9.35	16.10	36.42
NUM	95.90	173.92	37.47
CFW	5.22	12.23	43.95
SWL	18.79	25.94	23.48
Quality traits			
CWL	20.97	25.93	20.20
FLE	0.24	0.17	12.12
COL	80.78	16.56	3.49

[a] The units for each trait are explained in Table 1.
[b] The data are the averages for all the individuals tested, including three repetitions for each individual.

for each of the individuals tested. These procedures were applied to the map intervals defined by two consecutive markers present in a *P. ostreatus* genetic linkage map (20). SAS software (SAS Institute Inc., Cary, N.C.), particularly the procedure PROC NLIN, was used for computational analysis. This analysis provided estimates of the effect of a given possible QTL and information about its location in a particular interval. Multiple regression was used to calculate the percentage of the variance explained by each QTL, as estimated by the coefficient of determination (R^2). Two-way analysis of variance for a list of pairs of selected intervals with a significance threshold of $P < 0.005$ was applied in order to determine digenic interactions.

RESULTS

General analysis of the data. The values recorded for all the production and quality traits studied except precocity (P_{21} and P_{15}) were distributed normally (data not shown). For the statistical analysis, precocity data were not transformed because transformation did not improve the normality of their distribution. Table 2 summarizes average values for the traits, their variation ranges, and their variation coefficients. The variation coefficients were different for the different traits; however, the analysis of variance showed that the different genotypes had a significant effect on each of the traits analyzed (data not shown). Table 3 shows the correlation coefficients for all the pairs of production and quality traits analyzed.

Production characters. Fruiting occurred earlier at 21°C than at 15°C ($P_{21} < P_{15}$) (Table 2), and the average yield was higher at the higher temperature ($Y_{21} > Y_{15}$ and $FFY_{21} > FFY_{15}$) (Table 2). The correlation coefficients for the precocity (P_{21} and P_{15}) and yield (Y_{21} and Y_{15}) values at the two fruiting temperatures assayed were high (Table 3), indicating that there is a genetic component for these parameters. The precocity and yield values (P_{21} and Y_{21}; P_{15} and Y_{15}) were also highly correlated (Table 3), suggesting that precocity is a good predictor for culture yield. This was also the case when first-flush yield and total-yield values were compared, indicating that first-flush yield is a good predictor for total yield at both temperatures. In order to analyze the effect of the two temperatures tested on fruiting, the distribution of differences between the precocity and yield values for each individual measured at 15 or 21°C ($P_{15\times21}$ and $Y_{15\times21}$) was studied (Tables 2 and 3). Low levels of correlation between precocity ($P_{15\times21}$) or yield ($Y_{15\times21}$) differences and the other production parameters were found.

The total yield at 21°C was divided into two components: number of fruiting bodies and fruiting body average weight (Table 1). These two components were negatively correlated (Table 3), indicating that the higher the number of fruiting bodies, the lower the average weight. Negative correlation values were obtained for number of fruiting bodies and precocity, indicating that the most precocious individuals (lower precocity values) tended to produce a higher number of smaller fruiting bodies. The correlation coefficients for number of fruiting bodies or fruiting body average weight with yield were relatively low, although they were highly significant. In general, the correlation of yield was greater with number of fruiting bodies than with fruiting body average weight (Table 3).

Mapping of production traits. In order to identify the genomic regions involved in control of quantitative traits, a *P. ostreatus* var. *florida* genetic linkage map previously described by Larraya et al. (20) was divided into 109 intervals defined by pairs of consecutive map markers, and each interval was tested for the presence of a QTL effect on every trait. A segregating population of 80 individuals (population D80) (see Materials and Methods) was used to map all the production traits analyzed except $P_{15\times21}$ and fruiting body average weight, which were mapped by using a larger population consisting of 130 individuals (population D130). Table 4 summarizes the QTLs found, as well as their map positions and effects.

A total of 18 different QTLs that control the production traits discussed above were mapped to different linkage groups. These QTLs explained between 5.70% (*CFW-1*) and 48.36% (*FFY_{21}_2*) of the total variation of the different traits. A hot spot for QTLs that control production was found in linkage group VII (map position defined by marker *$L16_{875}$*), to which the main QTLs identified for each of the production traits studied mapped (Table 4 and Fig. 1). In some cases, the QTL was the only QTL that could be identified for some traits (P_{15}, Y_{15}). No QTLs could be detected for yield differences at the two culture temperatures ($Y_{15\times21}$), whereas two QTLs were detected for temperature-dependent precocity differences ($P_{15\times21}$). One of the latter QTLs (*$P_{15\times21}$_2*) mapped to the same position as the main production QTL located on chromosome VII. Two QTLs that control the number of fruiting bodies and two QTLs that control the fruiting body average weight were identified. In both cases, one of the QTLs (*NUM_2*, *CFW_2*) corresponded to the main production QTL described above.

Digenic interactions. The occurrence of digenic interactions that significantly affect ($P < 0.005$) precocity and yield at 15 and 21°C was studied by analyzing the effects of all possible allelic combinations in each gene pair on the traits. To do this, all possible pairs of genetic markers (i.e., QTLs detected in this assay, anonymous markers, and functional genes) present in the genetic linkage map were considered. A total of 14 functional genes, including the genes encoding four hydrophobins (*vmh1*, *vmh2*, and *vmh3* expressed in vegetative mycelium and

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TABLE 3. Correlation coefficients for quantitative traits

Trait	Correlation coefficient (significance)												
	P_{15}	Y_{21}	Y_{15}	FFY_{21}	FFY_{15}	$P_{15\times21}$	$Y_{15\times21}$	NUM	CFW	SWL	CWL	FLE	COL
P_{21}	0.87 (<0.01)	−0.79 (<0.01)	−0.74 (<0.01)	−0.66 (<0.01)	−0.64 (<0.01)	0.30 (<0.01)	0.04 (0.69)	−0.59 (<0.01)	0.45 (<0.01)	0.29 (<0.01)	0.15 (0.08)	0.04 (0.64)	−0.13 (0.15)
P_{15}		−0.77 (<0.01)	−0.80 (<0.01)	−0.70 (<0.01)	−0.67 (<0.01)	0.72 (<0.01)	−0.04 (0.64)	−0.58 (<0.01)	0.46 (<0.01)	0.21 (0.02)	0.16 (0.06)	0.09 (0.31)	−0.16 (0.06)
Y_{21}			0.67 (<0.01)	0.81 (<0.01)	0.62 (<0.01)	−0.40 (<0.01)	0.15 (0.09)	0.66 (<0.01)	−0.37 (<0.01)	−0.16 (0.07)	−0.26 (<0.01)	0.18 (0.04)	−0.07 (0.44)
Y_{15}				0.63 (<0.01)	0.86 (<0.01)	−0.49 (<0.01)	−0.27 (<0.01)	0.57 (<0.01)	−0.44 (<0.01)	−0.09 (0.33)	−0.13 (0.14)	−0.11 (0.23)	0.07 (0.42)
FFY_{21}					0.62 (<0.01)	−0.44 (<0.01)	0.36 (<0.01)	0.58 (<0.01)	−0.32 (<0.01)	−0.12 (0.16)	−0.09 (0.29)	0.05 (0.58)	0.05 (0.58)
FFY_{15}						−0.37 (<0.01)	−0.47 (<0.01)	0.53 (<0.01)	−0.36 (<0.01)	−0.14 (0.12)	−0.07 (0.44)	−0.07 (0.40)	−0.02 (0.83)
$P_{15\times21}$							−0.15 (0.10)	−0.31 (<0.01)	0.27 (<0.01)	0.01 (0.95)	0.11 (0.23)	0.12 (0.19)	−0.14 (0.11)
$Y_{15\times21}$								0.01 (0.87)	0.07 (0.44)	0.03 (0.77)	−0.02 (0.81)	0.14 (0.10)	0.08 (0.38)
NUM									−0.74 (<0.01)	0.24 (<0.01)	−0.09 (0.30)	−0.31 (<0.01)	−0.01 (0.93)
CFW										−0.41 (<0.01)	0.08 (0.39)	0.52 (<0.01)	−0.08 (0.40)
SWL											0.21 (0.02)	−0.31 (<0.01)	−0.11 (0.19)
CWL												−0.27 (<0.01)	0.07 (0.41)
FLE													−0.20 (0.03)

fbh1 expressed in the fruiting body), two lacasses (*pox1* and *poxC*), a veratryl alcohol oxidase (*vao*), and three manganese peroxidases (*mnp1*, *mnp2*, and *mnp3*), three mating type genes (*matA*, *matB*α, and *matB*β), and the ribosomal DNA gene (*rib*), which mapped to different sites in the whole *P. ostreatus* genome, were considered. A number of significant digenic interactions affecting precocity and yield at 15 and 21°C were detected (Table 4). They included interactions between QTLs and anonymous markers (Table 5, interactions 1 to 3, 5, 11, and 25), between anonymous markers (Table 5, interactions 4, 6 to 10, 12, 14 to 16, 18, 19, 21 to 24, and 26 to 39), and between anonymous markers and functional genes (Table 5, interactions 13, 17, and 20). Some interactions could be detected for both traits at 15°C (Table 5, compare interaction 15 with interaction 38 and interaction 18 with interaction 31) or at 21°C (Table 5, interactions 10 and 21), and others were detected for a trait at both temperatures (Table 5, interactions 13 and 17). Finally, some anonymous markers appeared to be involved in digenic interactions that affected three ($P12_{950}$, $L6_{1800}$) or all ($R7_{2400}$, $P4_{1450}$, $R14_{700}$) of the traits analyzed (P_{21}, P_{15}, Y_{21}, and Y_{15}). All the significant digenic interactions that affect precocity and yield are shown in Fig. 1.

Quality characteristics. The four traits related to mushroom quality analyzed for mushrooms grown at 21°C (Table 1) had, in general, low correlation coefficients with each other and with the production traits (Table 3). A total of 10 QTLs that control quality traits were identified. To map most of them the enlarged D130 population was used; the only exception was stipe weight loss, for which the D80 segregating population was used. In contrast to QTLs that control production, the quality QTLs identified appeared to be scattered across the *P. ostreatus* genome, and they explained a smaller portion of the total variation of the traits (Table 4 and Fig. 1).

Production and quality performance of the new hybrids. The values for the production and quality traits studied were recorded for the hybrids belonging to population D130. Table 6 indicates the best and worst 15 performances for some of the traits recorded in the experiment performed at 21°C. For comparison, two commercial strains (strains 200 and 201) were included in the study. For one trait (P_{21}) commercial strain 200 performed better than the strains constructed in this work, but for the other traits the newly constructed hybrids performed better (in Table 6 all strains in the lists above strain 200 or 201 produced preferable values).

DISCUSSION

Study of the genetic basis of quantitative traits can be approached by comparing the phenotypes displayed by different genotypic classes defined by single loci or by genomic intervals limited by two genetic markers. In the present study, we mapped QTLs that control agronomic and industrial traits to chromosome intervals defined by molecular markers whose positions in the genetic linkage map of *P. ostreatus* var. *florida* are known (20). This approach has been successfully used previously for mapping QTLs that control the mycelial monokaryotic and dikaryotic growth rates in *P. ostreatus* (17); in this study, however, we concentrated on quantitative traits of major interest for large-scale production of this edible mushroom, and we tried to develop a basis for a genomic approach to

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TABLE 4. QTLs for quantitative traits

Trait	Mapping population	QTL	Linkage group	Position[a]	Probability	Absolute effect[b]	R^2	Total R^2
Production traits								
P_{21}	D80	P_{21}_1	I	$L18_{2175}$ + 6.7	0.02713	2.31	8.70	
		P_{21}_2	VI	*poxC* + 0	0.01661	2.10	9.67	
		P_{21}_3	VII	$LI6_{875}$ + 0	<0.00001	3.77	31.83	
		P_{21}_4	VIII	$R20_{1950}$ + 16	0.03116	2.28	9.25	47.96
P_{15}	D80	P_{15}_1	VII	$L16_{875}$ + 0	<0.00001	5.99	36.30	36.30
$P_{15\times21}$	D130	$P_{15\times21}$_1	I	$S12_{2075}$ + 2	0.00003	2.11	15.54	
		$P_{15\times21}$_2	VII	$L16_{875}$ + 0	<0.00001	2.46	22.68	32.05
Y_{21}	D80	Y_{21}_1	IV	*mnp1* + 0.1	0.01125	2.63	11.70	
		Y_{21}_2	VII	$L16_{875}$ + 0	<0.00001	5.09	45.47	47.69
Y_{15}	D80	Y_{15}_1	VII	$L16_{875}$ + 0	<0.00001	4.50	32.07	32.07
FFY_{21}	D80	FFY_{21}_1	IV	*mnp1* + 0	0.03049	2.09	9.09	
		FFY_{21}_2	VII	$L16_{875}$ + 0	<0.00001	4.69	48.36	49.26
FFY_{15}	D80	FFY_{15}_1	VI	*poxC* + 3.8	0.02707	2.24	9.94	
		FFY_{15}_2	VII	$L16_{875}$ + 0	<0.00001	3.86	28.77	34.28
NUM	D80	*NUM_1*	IV	$R9_{1300}$ + 1.5	0.00501	29.30	12.75	
		NUM_2	VII	$L16_{875}$ + 0	0.00010	33.78	21.71	27.57
CFW	D130	*CFW_1*	III	*matA* + 0	0.02021	1.24	5.70	
		CFW_2	VII	$L16_{875}$ + 0	0.00035	1.90	13.83	18.13
Quality traits								
SWL	D80	*SWL_1*	I	$L18_{2175}$ + 0	0.04635	2.78	3.73	
		SWL_2	II	$R20_{1400}$ + 0.1	0.01152	3.64	11.14	14.27
CWL	D130	*CWL_1*	V	$S7_{1200}$ + 0	0.04164	1.90	4.49	
		CWL_2	VI	$L1_{1075}$ + 0	0.00687	2.48	7.89	
		CWL_3	VIII	$S11_{2325}$ + 0	0.00513	2.79	7.67	19.43
FLE	D130	*FLE_1*	III	*matA* + 0	0.00175	0.019	10.19	
		FLE_2	V	$S19_{2600}$ + 0	0.02309	0.014	5.14	
		FLE_3	VI	$L1_{1075}$ + 0	0.00168	0.019	10.16	26.87
COL	D130	*COL_1*	IV	$R9_{1300}$ + 5	0.00161	2.23	9.93	
		COL_2	X	$R4_{1175}$ + 0	0.00682	1.58	7.26	17.31

[a] Map position of QTLs at a marker locus plus the distance (in centimorgans) towards the bottom of the linkage group (Fig. 1).
[b] Estimated absolute difference between alleles at the QTL, expressed as described in Materials and Methods.

fungal breeding of new industrial strains. The analytical procedure used here can be applied to other fungal systems for identification of genome regions involved in the control of complex phenotypic traits.

Ten production traits and four quality traits were studied in this work (Table 1), and QTLs that control them were found in all *P. ostreatus* chromosomes except chromosomes IX and XI (Fig. 1). In general, the quality traits analyzed had lower variation coefficients than the production traits had (Table 2). This fact, along with the strong influence of the environment on quality traits, reduced the precision of QTL detection (reflected in low total R^2 values [Table 4]). However, quality QTLs showed a high level of additivity in the individual effects, resulting in a total R^2 value similar to the sum of the partial values. Detection of QTLs that control traits with low variability was more effective when a larger mapping population (D130) was used. This indicates that the mapping population size seems to be more important than the degree of linkage map saturation in order to detect QTLs for this kind of trait.

Mapping of production QTLs in *P. ostreatus* N001 revealed the occurrence of a genomic region in chromosome VII that is highly significant in genetic control of this trait (Fig. 1). Moreover, in a previous study (17) two QTLs that control the dikaryotic growth rate in Eger medium (namely, *Qdgre5* and *Qdgre11*), which were discovered by using a common nucleus (tester) different from PC21, were mapped to this position. No candidate genes can be suggested for this genome region since only one putative functional gene has been mapped to it so far. When molecular marker $L16_{875}$ is used as the query in a BlastX similarity search, a sequence coding for *Aspergillus aculeatus* β-rhamnosidase is found (22). Whether this gene function is relevant for the QTL activity found is not clear yet. In addition, it should be noted that chromosome VII presents an important length polymorphism in strain N001 (19). The effects of the two nuclei present in *P. ostreatus* strain N001 can be studied in protoclones PC9 and PC15 (19), which have different morphological and biochemical characteristics. Protoclone PC15 carries the larger chromosome VII homologue and has a lower mycelial growth rate and higher cellulolytic activity than protoclone PC9. In population D80 (produced by mating the monokaryons of segregating population M80 to the compatible monokaryon PC21 [see Materials and Methods]), the dikaryons harboring the larger chromosome VII produced a higher yield than the dikaryons bearing the smaller chromosome produced. No correspondence between the QTLs that control the growth rate previously described (17) and the QTLs described in this work has been found so far. Hence, the possibility of a relationship between chromosome size and production characteristics in *P. ostreatus* deserves more thoughtful study.

Precocity and yield traits are correlated, indicating that precocious genotypes are in general the most productive genotypes (Table 3) (notice that precocity is estimated by determining the time that elapses until the first harvest). Thus, the yield could be related to the cropping period because the most

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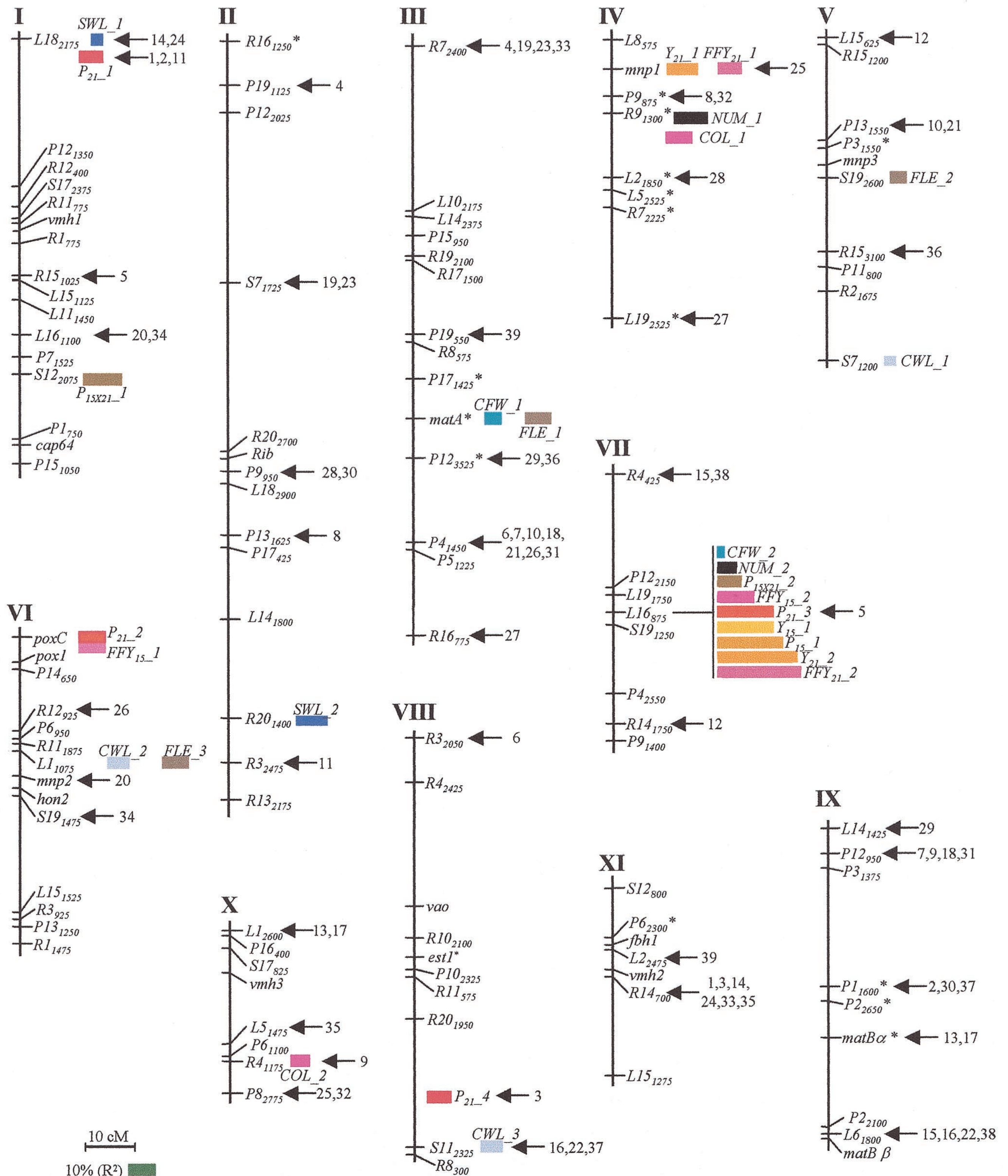

FIG. 1. Genetic linkage map of *P. ostreatus* (20), including the positions of QTLs and digenic interactions for production and quality traits. QTLs are placed at their approximate positions to the right of the markers. The percentages of the phenotypic variation explained by the putative QTLs (R^2) are represented by proportional boxes. Significant interactions among QTLs, genes, and other molecular markers are indicated by arrows and numbers (see Table 5). Randomly amplified polymorphic DNA markers in the map are indicated by the primer name and the approximate length of the corresponding amplification product. Genes are in italics. Markers that deviated from the expected 1:1 segregation ($P < 0.05$) are indicated by an asterisk.

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TABLE 5. Digenic interactions for precocity and yield at 15 and 21°C

Interaction	Interaction term	Map position	Probability	Means for the following genotypic classes[a]:			
				00	01	10	11
Interactions with P_{21}							
1	$P_{21}_1 \times R14_{700}$	I-XI	0.00044	10.29	6.95	5.72	7.49
2	$P_{21}_1 \times P1_{1600}$	I-IX	0.00054	11.33	7.43	5.96	7.36
3	$P_{21}_4 \times R14_{700}$	VIII-XI	0.00063	6.32	10.72	7.60	6.90
4	$P19_{1125} \times R7_{2400}$	II-III	0.00065	5.91	9.03	8.71	6.36
5	$R15_{1025} \times P_{21}_3$	I-VII	0.00209	5.56	11.28	6.53	8.26
6	$P4_{1450} \times R3_{2050}$	III-VIII	0.00224	6.75	8.05	9.61	6.21
7	$P4_{1450} \times P12_{950}$	III-IX	0.00247	8.00	6.94	6.18	10.27
8	$P13_{1625} \times P9_{875}$	II-IV	0.00263	6.81	8.04	9.94	6.30
9	$P12_{950} \times R4_{1175}$	IX-X	0.00338	7.93	6.54	6.08	9.19
10	$P4_{1450} \times P13_{1550}$	III-V	0.00373	6.68	8.61	9.31	6.61
11	$P_{21}_1 \times R3_{2475}$	I-II	0.00390	10.39	6.56	6.58	6.90
12	$L15_{625} \times R14_{1750}$	V-VII	0.00398	7.11	5.51	7.92	10.5
13	$matB\alpha \times L1_{2600}$	IX-X	0.00444	9.35	6.98	5.72	7.77
Interactions with P_{15}							
14	$L18_{2175} \times R14_{700}$	I-XI	0.00177	15.23	11.19	9.33	12.33
15	$R4_{425} \times L6_{1800}$	VII-IX	0.00177	13.69	10.87	9.33	13.67
16	$S11_{2325} \times L6_{1800}$	VIII-IX	0.00244	9.09	15.47	11.99	11.57
17	$matB\alpha \times L1_{2600}$	IX-X	0.00258	13.73	10.80	9.14	13.29
18	$P4_{1450} \times P12_{950}$	III-IX	0.00402	13.00	11.16	9.65	14.46
19	$S7_{1725} \times R7_{2400}$	II-III	0.00416	8.93	12.17	13.49	10.46
20	$L16_{1100} \times mnp2$	I-VI	0.00481	13.48	9.42	11.33	13.71
Interactions with Y_{21}							
21	$P4_{1450} \times P13_{1550}$	III-V	0.00079	20.17	17.24	17.28	20.33
22	$S11_{2325} \times L6_{1800}$	VIII-IX	0.00177	21.26	17.39	18.19	19.73
23	$S7_{1725} \times R7_{2400}$	II-III	0.00205	20.98	18.79	17.41	20.99
24	$L18_{2175} \times R1_{4700}$	I-XI	0.00276	16.02	18.84	21.31	18.78
25	$Y_{21}_1 \times P8_{2775}$	IV-X	0.00323	19.10	19.03	16.08	21.18
26	$P4_{1450} \times R12_{925}$	III-VI	0.00336	17.11	21.21	19.14	18.33
27	$R16_{775} \times L19_{2525}$	III-IV	0.00393	17.23	20.21	20.09	17.83
28	$P9_{950} \times L2_{1850}$	II-IV	0.00453	20.16	18.82	15.84	19.91
29	$P12_{3525} \times L14_{1525}$	III-IX	0.00492	18.55	19.95	20.01	16.07
30	$P9_{950} \times P1_{1600}$	II-IX	0.00496	17.71	20.10	20.25	17.58
Interactions with Y_{15}							
31	$P4_{1450} \times P12_{950}$	III-IX	0.00004	10.86	14.20	15.17	11.21
32	$P9_{875} \times P8_{2775}$	IV-X	0.00068	13.92	11.84	11.03	15.37
33	$R7_{2400} \times R14_{700}$	III-XI	0.00189	15.39	11.78	11.38	13.42
34	$L16_{1100} \times S19_{1475}$	I-VI	0.00212	12.08	15.24	13.66	11.26
35	$L5_{1475} \times R14_{700}$	X-XI	0.00258	14.63	11.88	11.03	13.77
36	$P12_{3525} \times R15_{3100}$	III-V	0.00281	12.98	11.45	12.21	16.73
37	$S11_{2325} \times P1_{1600}$	VIII-IX	0.00285	15.69	10.59	12.50	13.00
38	$R4_{425} \times L6_{1800}$	VII-IX	0.00477	11.93	13.64	14.99	11.56
39	$P19_{550} \times L2_{2475}$	III-XI	0.00489	14.64	11.67	12.01	14.29

[a] Phenotypic means for genotypic classes 00, 01, 10 and 11 were obtained by using the two markers involved in the digenic interaction (0, marker absent; 1, marker present).

precocious genotype is probably the most productive genotype in the first month of cropping. In support of this hypothesis, the correlation coefficient for precocity and first-flush yield is lower than the correlation coefficient for precocity and total yield (Table 3). Analysis of the yield components showed that there were significant correlations among the three variables (total yield, number of fruiting bodies, and fruiting body average weight). The yield and fruiting body average weight were negatively correlated, whereas the yield and number of fruiting bodies were positively correlated. Similar results have been obtained for *A. bisporus*, another edible mushroom crop (26).

A notable genotype-environment interaction can be proposed on basis of the QTLs and digenic interactions detected at different temperatures. When the yield was assayed at 15 and 21°C, different significant regions appeared to be located near lignin-degrading enzymes, *mnp1* and *poxC* (Fig. 1 and Table 4). This fact could suggest that there is a differential temperature dependence for these activities. Besides the different responses of production traits to the fruiting temperature, the tolerance of genotypes to this factor was estimated in this study. For this trait, a new QTL located in linkage group I ($P_{15\times21}$) seems to be partially responsible for the fruiting ability at different temperatures (Fig. 1 and Table 4).

There have been only a limited number of reports dealing with directed breeding of *P. ostreatus* strains (2, 21, 25). This study provided the first opportunity for molecular marker-assisted selection of new *P. ostreatus* strains. Considering that all the dikaryons of the mapping population share one identical nucleus (the tester nucleus provided by protoclone PC21), the QTLs identified in this study reflect the effect of the segregating nuclei plus their interaction with the constant tester nucleus. These interactions are variable in terms of the func-

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TABLE 6. Production and quality performance of selected traits of the hybrids at 21°C[a]

Category	P_{21}		Y_{21}		FFY_{21}		CFW		SWL		CWL		FLE	
	Strain[b]	Days	Strain[b]	%	Strain[b]	%	Strain[b]	Avg	Strain[b]	%	Strain[b]	%	Strain[b]	Amt (g/cm^2)
Best scores	**200**	**3.00**	139	25.10	139	18.18	55	14.70	97	9.56	159	13.32	105	0.33
	35	4.00	147	24.47	137	16.75	105	14.13	57	9.61	135	13.86	57	0.30
	84	4.00	84	24.38	74	16.40	45	13.49	7	11.35	**200**	**13.92**	132	0.30
	110	4.00	107	24.08	107	16.05	112	11.85	55	11.43	109	14.02	84	0.29
	139	4.00	46	23.97	109	15.62	7	9.78	45	11.82	119	14.11	93	0.29
	160	4.00	24	23.72	2	15.30	93	9.52	27	12.05	121	14.79	138	0.29
	177	4.00	**200**	**23.52**	177	15.30	57	9.52	42	12.17	33	14.85	112	0.29
	201	**4.00**	10	23.40	27	15.08	87	9.45	69	12.28	46	14.94	10	0.29
	88	4.33	109	23.37	6	15.03	97	9.19	35	13.45	169	14.96	154	0.28
	109	4.33	27	23.05	100	14.95	27	9.16	118	13.57	62	15.30	27	0.28
	147	4.33	74	22.92	126	14.85	20	8.68	93	13.64	162	15.37	85	0.28
	148	4.33	22	22.68	110	14.75	176	8.45	138	13.65	84	15.41	46	0.28
	152	4.33	177	22.67	90	14.73	138	8.19	94	13.66	129	15.61	173	0.28
	159	4.33	88	22.63	29	14.68	122	8.09	123	13.85	24	15.96	125	0.28
	24	4.67	135	22.53	72	14.58	16	7.85	6	14.09	4	16.06	17	0.28
Worst scores	115	10.67	21	14.48	8	7.13	53	3.16	98	24.39	126	25.45	160	0.20
	149	10.67	61	14.37	128	7.12	107	3.15	30	24.53	97	25.68	128	0.20
	9	11.00	70	14.37	149	7.00	91	3.13	21	24.55	139	25.77	174	0.20
	11	11.00	8	14.30	20	6.92	35	3.08	9	24.67	1	26.09	50	0.20
	14	11.00	133	14.22	48	6.92	159	3.08	154	24.73	175	26.43	59	0.20
	25	11.00	7	14.02	59	6.63	**201**	**2.92**	130	24.73	11	27.03	155	0.20
	85	11.00	62	13.73	67	6.62	14	2.86	31	24.81	67	27.91	152	0.20
	73	12.33	58	13.47	169	6.42	59	2.83	157	25.01	122	28.27	21	0.20
	157	14.33	55	13.18	154	6.07	72	2.70	139	25.24	37	28.34	166	0.20
	41	16.00	41	12.55	7	5.85	129	2.69	105	25.77	146	28.38	98	0.19
	62	16.00	15	12.28	58	5.03	150	2.62	59	26.46	176	29.11	127	0.19
	138	18.33	16	10.97	31	4.50	160	2.60	14	27.64	150	30.00	67	0.19
	55	19.00	138	8.55	85	4.07	**200**	**2.57**	26	29.76	124	30.94	1	0.19
	105	19.33	105	8.10	15	4.05	174	2.49	115	31.55	61	33.57	30	0.18
	64	21.67	64	7.78	16	3.57	130	2.48	64	35.50	115	39.24	150	0.16

[a] Traits are defined in Table 1.
[b] Strain numbers correspond to the numbers of the monokaryons of population D130. Strains 200 and 201 (indicated by boldface type) are the commercial strains used as reference strains for comparison.

tion of the tester used (17). The tester nucleus (PC21) belonged to *P. ostreatus* var. *ostreatus*, whereas the segregating monokaryotic population was derived from *P. ostreatus* var. *florida*. The two varieties, which are commonly used for production of commercial hybrids, have different origins and differ in several characteristics. *P. ostreatus* var. *florida* type is smaller, has a finer structure and lighter color, is more tolerant to high temperatures during fruiting, and produces a higher yield than *P. ostreatus* var. *ostreatus* (8, 27). However, both varieties kept a high degree of synteny when the progeny were analyzed by using the molecular markers described previously (Larraya et al., unpublished results). Taking into account this information, it should be possible to use molecular marker-assisted selection for identification of hybrid strains having the optimal combination of the traits analyzed.

In the course of the experiments described in this paper, we constructed a number of new hybrid strains that were used to identify the genomic regions involved in an increase in production and/or quality. Although the new strains were not formally designed after marker-assisted selection of the parental strains, they were used as the basis for identification of markers that can be used in such a marker-assisted selection process. Furthermore, some of the new strains showed productivity or quality values higher than those of the parental strains or other commercial strains

However, there is a theoretical issue in this context. It is difficult to predict the result of a mating between two siblings containing QTL variants responsible for high productivity or quality because the dikaryotic nature of higher basidiomycetes makes the genetic control of gene expression different than the genetic control found in normal diploids. In particular, it is not known if phenomena such as inbreeding depression and positive or negative heterosis occur in dikaryotic systems. These kinds of questions can be addressed now that there is a system in which QTLs for production traits are available.

A final step in understanding gene expression regulation and genome organization is identification of candidate genes and analysis of the genetic factors controlling characteristics that exhibit continuous variation (15, 24). In this context, the search for candidate genes in the genomic region bearing the major production QTLs on chromosome VII and the study of some regions on chromosomes III, IX, and XI involved in multiple digenic interactions are appealing. Mating of the collection of monokaryons with other compatible testers should shed light on the influence of these interactions on quality and production traits.

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