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GENETIC VARIATION OF MF (ALPHA) 1 GENE AMONG SACCHAROMYCES CEREVISIAE POPULATION

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ABSTRACT

The fully sequenced genomes of five species within the Saccharomyces sensustricto complex provided a wealth of information for molecular-evolutionary inference. Yet virtually little thing was known about population-genetic variation within these species, including the molecular-biological and genetic-model organism S. cerevisiae. Here we investigated the population genetic variation and population structure of S. cerevisiae by analyzing the sequence of the MF(alpha)1 loci in 41 strains. Sequence analysis demonstrated a distinct population structure in S. cerevisiae, distinguishing strain collected from Kunming grape juice and other strains. Our findings indicated that strains clustered together might mainly due to ecological rather than geographic factors.

Keywords: S. cerevisiae, Strains, MF(alpha)1, Genetic variation.

1. INTRODUCTION

Yeast is important to human beings, yeast strains have been used to make bread, wine, beer, spirits and sake. In recent times, yeast strains have been used as a model organism in scientific studies and also for ethanol production from sugar and grain [1]. Yeast has been shown to have a lot of biodiversity genotypically and phenotypically [2]. The huge diversity of yeast strains coupled with breeding strategies would contribute greatly to improving industrial yeasts [2, 3]. DNA sequencing studies conducted by Fay and Benavides [4] and Aa, et al. [5] also found that there was a lot of diversity between different strains. This technique has previously been used to differentiate between different commercial yeast strains [6, 7].

In heterothallic fungi, mating occurs only between haploid cells of opposite mating types; the mating is initiated by cell-specific pheromone and receptor combinations [8, 9]. The specific recognition launches a complex mitogen-activated protein kinase (MAPK) cascade, the pheromone

response pathway. The MF(alpha)1 gene of *Saccharomyces cerevisiae* was one of the best-studied pheromone precursor genes which had been identified in the fungi [10]. Mating pheromone alpha-factor, made by alpha cells; interacts with mating type a cells to induce cell cycle arrest and other responses leading to mating; also encoded by MF(alpha)2, although MF(alpha)1 produces most alpha-factor [11]. Pheromone precursor genes have been identified throughout the fungal kingdom [12, 13]. In addition to *S. cerevisiae*, other heterothallic ascomycetes also contain pheromone precursor genes, as do heterothallic basidiomycetes, homobasidiomycetes, and a homothallic ascomycete [14].

Here,Genetic variation of MF(alpha)1 gene among*S.cerevisiae* population were presented.

2. MATERIAL AND METHODS

2.1. Samples

S.cerevisiae isolate was obtained by direct plating fromgrape juiceof Kunming, Yunnan, China, after dilution as appropriate, on YPD plates [15] containing chloramphenicol (100 g/mL).

2.2. DNA Extraction

DNA from yeast was isolated using 5% Chelex solution [16]. A single colony was picked with a sterile pipette tip and dispersed in 5% Chelex, followed by vortexing for 15 seconds, after which the mixture was boiled at 100℃ for 10 minutes. The DNA mixture was then vortexed for another 15 seconds and centrifuged at 13000 *g*for 3 minutes. The supernatant was stored at -20℃.

2.3. PCR

PCR reaction mixtures (25 μL) were set up with the following components: 2.5 μL 10 × PCR buffer (including Mg^{2+}), 1 μL each of 10 mM primers, 0.5 μL of 10 mM of each dNTP, 0.2 μL of 5U/μL*Taq* DNA polymerase, 2 μL of template DNA and dH_2O up to 25 μL. Programmes used forPCR were:5 minutes of 95℃; followed by 35 cycles of 30 seconds of 95℃, 30 seconds of 50℃ annealing and 60 seconds of 72℃; followed by 7 minutes of 72℃. The sequences of the primers were sMFG1-U: AAA GCA ACA ACA GGT TTT GG and sMFG1-L: CAA ATT GAA ATA TGG CAG GC. Yields of PCR products were assayed by agarose gel electrophoresis. Fragments were separated on 1% or 3% agarose gels made in 1× TBE (Tris-borate-EDTA) buffer. Gels were stained with ethidium bromide, visualized, and photographed under UV light. Fragment sizes were estimated by comparison against a DNA standard (1Kb plus ladder; Invitrogen).

2.4. Purification of PCR Products and Sequencing

PCR products for sequencing were purified using High Pure PCR Product Purification Kit (Roche) according to the manufacturer's instructions and analyzed on an Applied Biosystems DNA Sequencing 3130XL machine.

2.5. Sequence Analysis

Sequences were edited using Vector NT, transformed into FASTA format and compared to

the NCBI database using BLAST. The relevant sequences for the international *S.cerevisiae* strains generated by Liti, et al. [17] were obtained from http://www.sanger.ac.uk/research/faculty/rdurbin/sgrp.html and NCBI database. The evolutionary distances were computed using the Maximum Composite Likelihood method [18] and were in the units of the number of base substitutions per site. Substitution pattern and rates were estimated under the Kimura [19] 2-parameter model. Codon positions included were 1st+2nd+3rd+ Noncoding. All positions containing gaps and missing data were eliminated. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). There were a total of 578 positions in the final dataset. The analysis involved 41 nucleotide sequences. Evolutionary analyses were conducted in MEGA5 [20].

3. RESULTS AND DISCUSSION

3.1. Structure of Predicted MF (Alpha) 1 Gene

A 778 base fragment was amplified and sequenced. ORF Number 1 on the direct strand extended from base 1 to base 561. ORF Number 2 on the direct strand extended from base 625 to base 735. The mature α-factor was unmodified and hydrophilic. Normally, MF (alpha)1 gene encodes a precursor containing multiple repeats of a pheromone sequence bordered by Kex2-protease processing sites (Figure 1). The mature α-factor mating pheromone is highly hydrophobic due to prenylation at the cysteine residue, which is also carboxymethylated. These critical features of pheromone precursor genes are conserved in other fungi.

Figure- 1. Structure of predicted MF (alpha)1 gene from *S. cerevisiae* of Kunming strain.

The nucleotide sequence and predicted amino acid sequence of the α-factor precursors were shown. A putative motif was shown, and the predicted mature peptide was highlighted under gray nucleotide sequence. A potential cleavage site for the metaloprotease was indicated.

3.2. Transitional and Transversionsal Substitutions

Given the availability of sequence data for overseas isolates of *S.cerevisiae*, Maximum Likelihood Estimate of Substitution Matrix was calculated. There were a total of 578 positions in the final dataset. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories, [+*G*], parameter = 0.1939). Rates of different transitional substitutions were shown (Table 1) in bold and those of transversionsal substitutions were shown in *italics*. Relative values of instantaneous *r* should be considered when evaluating them. For simplicity, sum of *r* values is made equal to 100. The nucleotide frequencies were A = 25.00%, T/U = 25.00%, C = 25.00%, and G = 25.00%. For estimating ML values, a user-specified toplogy was used. The

maximum Log likelihood for this computation was -680.054. The estimated Transition/Transversion bias (*R*) was 7.47.

Table-1. Maximum Likelihood Estimate of Substitution Matrix.

	A	T/U	C	G
A	-	1.48	1.48	22.05
T/U	1.48	-	22.05	1.48
C	1.48	22.05	-	1.48
G	22.05	1.48	1.48	-

Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution was also computed. Rates of different transitional substitutions were shown (Talbe 2) in bold and those of transversionsal substitutions were shown in *italics*. The nucleotide frequencies were 31.81% (A), 26.18% (T/U), 22.35% (C), and 19.66% (G). The transition/transversion rate ratios were k_1 = 15.342 (purines) and k_2 = 7.337 (pyrimidines). The overall transition/transversion bias was R = 5.124, where $R = [A*G*k_1 + T*C*k_2]/ [(A+G)*(T+C)]$. And nucleotide pair frequencies among the 40 accessions were shown in table 3.

Table-2. Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution.

	A	T	C	G
A	-	1.09	0.82	18.2
T	1.33	-	20.39	0.93
C	1.33	27.14	-	0.93
G	25.91	1.09	0.82	-

Table-3. Nucleotide pair frequencies among the 41 accessions.

Domain		ii	si	sv	R	TT	TC	TA	TG	CT	CC	Domain Info
	Avg	438.00	10.00	4.00	2.84	107.00	3.00	0.00	0.00	2.00	88.00	Data
	1st	143.00	6.00	2.00	2.85	41.00	1.00	0.00	0.00	1.00	24.00	1st PosData
	2nd	146.00	3.00	1.00	3.65	32.00	1.00	0.00	0.00	1.00	28.00	2nd PosData
	3rd	149.00	1.00	1.00	2.02	34.00	0.00	0.00	0.00	0.00	36.00	3rd PosData
Domain	CA	CG	AT	AC	AA	AG	GT	GC	GA	GG	Total	Domain Info
	1.00	1.00	0.00	1.00	139.00	3.00	0.00	0.00	3.00	103.00	451.74	Data
	0.00	1.00	0.00	0.00	49.00	2.00	0.00	0.00	2.00	29.00	151.02	1st PosData
	0.00	0.00	0.00	0.00	36.00	0.00	0.00	0.00	0.00	50.00	149.69	2nd PosData
	0.00	0.00	0.00	0.00	54.00	0.00	0.00	0.00	0.00	24.00	151.03	3rd PosData

Note: All frequencies are averages (rounded) over all taxa; ii = Identical Pairs; si = Transitionsal Pairs; sv = Transversional Pairs; R= si/sv.

3.3. Evolutionary Relationships of the MF (Alpha) 1 Gene of Strains

Genetic variations of MF (alpha) 1 gene among the accessions were obvious (Figure 2). High polymorphism in the MF (alpha)1 gene provides evidence of diversifying selection on its protein produce. The evolutionary history was inferred using the Neighbor-Joining method [21]. UWOPS05_217_3 which got from nectar of bertram pine (in Malaysia), was inferred as an original sequence. The optimal tree with the sum of branch length = 0.21770316 was shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches [22]. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The over all average composition distances is 0.013. Pairwise distances ranged from 0 to 0.181. The current understanding is that *S. cerevisiae'*spopulaion is generally ecologically partitioned [5]. Our findings seemed to support this opinion. YPS128 and YPS606 both isolated in Pennsylvania (USA) fromOak shared a very closely relationship, and YJM978, YJM981 and YJM975, which were isolated in Bergamo (Italy) from vagin as clinical isolates, also shared closely relationships (Figure 3).

Figure-2. Portion of Aligment of MF (alpha)1 genes from 40 *S. cerevisiae* strains.

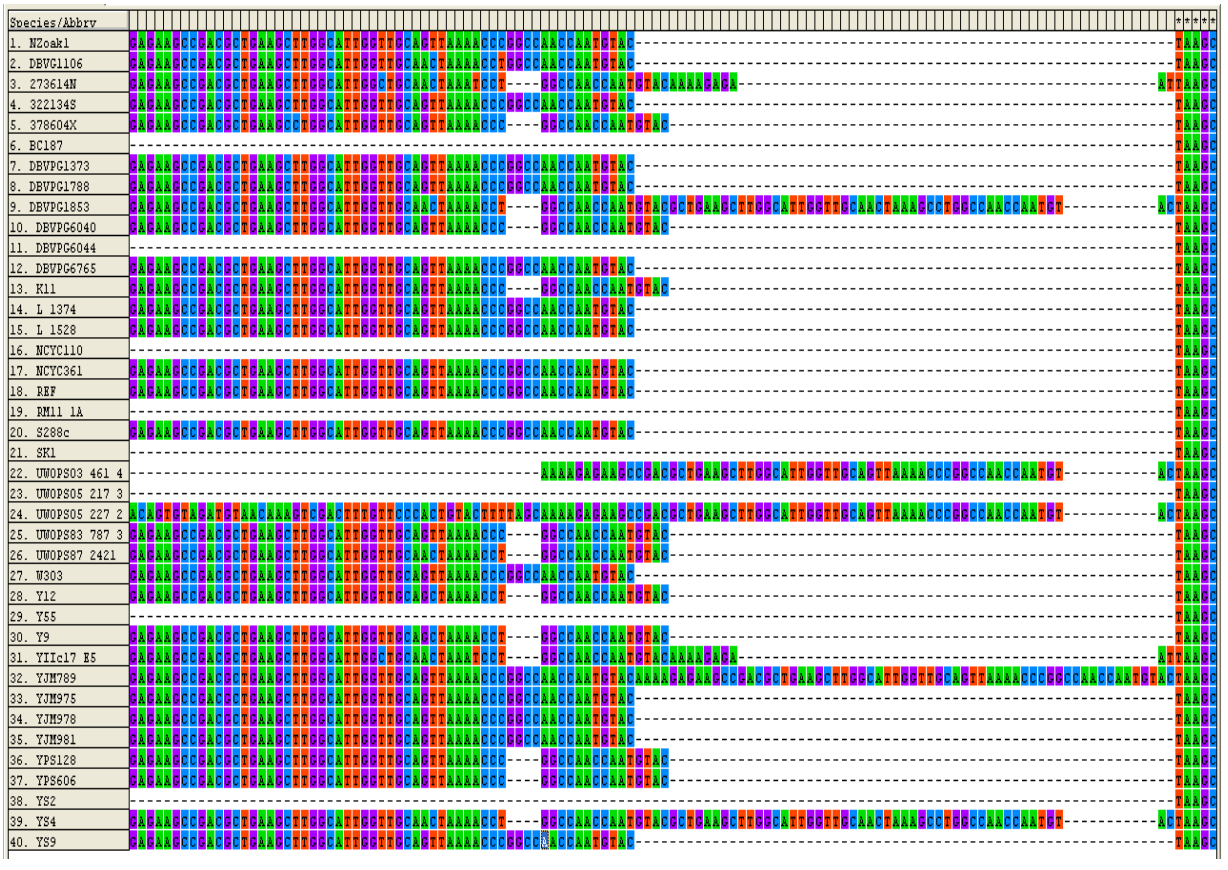
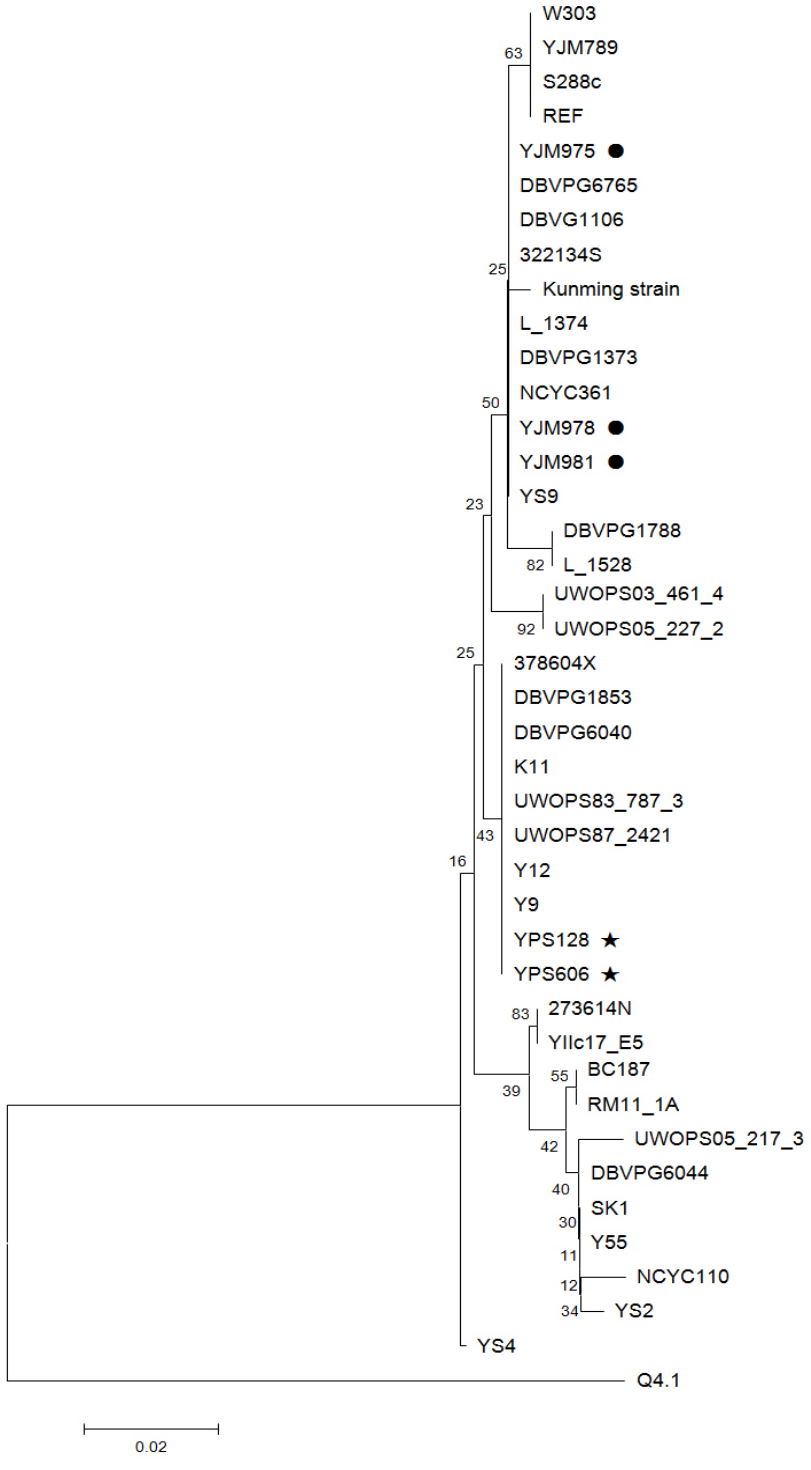


Figure-3. Evolutionary relationships of *S. cerevisiae* strains based on MF (alpha) 1 sequences.



Note: Q4.1 was a *S. paradoxus* strain, which was treated as an outgroup accession. Symbols indicate ecological origin: ●=Clinical isolate (vaginal); ★=Oak isolate.

The best hit of Kunming strain MF(alpha)1 gene was CBS432, which comes from Russia, then CBS 5829 (Denmark), then T8.1 (UK). In addition, the ITS sequence of *S. cerevisiae* were 100% identical to the European ones. It indicated that this *S. cerevisiae* strain might original from Europe. Since MF(alpha)1 and ITS sequences of the *S. cerevisiae* isolates showed that they were very closely related to European isolates, we hypothesized that they were introduced into Kunming Yunnan from Europe along with their host grape trees.

4. CONCLUSIONS

These data show that the population genetic variation and population structure of S. cerevisiae by analyzing the sequence of the MF(alpha)1 loci in 41 strains.

This is the first report of natural S. cerevisiae strain found in Kunming, Yunnan. And S. cerevisiae strains might be introduced into Kunming Yunnan from Europe along with their host grape trees.

Our findings indicated that strains clustered together might mainly due to ecological rather than geographic factors.

5. ACKNOWLEDGEMENTS

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