Sex-determining regions as drivers for evolutionary potential and phenotypic plasticity in Zygosaccharomyces rouxii clade

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State of the Art and Aim of the Work

In the allotriploid ATCC 42981 genome, MATα1 and MATα2 genes are from two different parents (Z. rouxii and Z. sapor, respectively). The different evolutionary history of α1 and α2 subunits could generate negative epistasis accounting for ATCC 4981 inability to repress HO gene and to enter into meiosis. To verify that the chimeric α1-α2 heterodimer is responsible for ATCC 4981 sterility, we planned to selectively replace Z. sapor MATα of ATCC 42981 with the orthologous Z. rouxii MATα.

Main Activities

1. Development of genetic toolkits and optimization of the electroporation protocol for the allotriploid ATCC 42981. Targeted deletion of Z. sapor MATα using a loxP-kanMX-loxP disruption system. Screening of deletion mutants for the ability to undergo meiosis and to survive stress.
2. Genome assemblies and functional genomics of ATCC 42981 and Z. sapor ABT301.2.
3. Discovering the a to a genotype switching in two different stocks of Z. rouxii CBS 732. Reconstruction of this mechanism that led to a new MATα2 gene. Investigation of the expression profile of HO endonuclease and analysis of the morphological and mating behavior of switched cultures.

1.1 Optimization of the Electroporation Protocol

Protocol for Z. rouxii transformation from Prybilova and Sychorova (2003) was optimized to efficiently introduce plasmid DNA into ATCC 42981 by electroporation, using plasmid pGPR2-2.2-phIurinin+ carrying NAP cassette and two pH-sensitive ratiometric phIurin3s.

1.2 Targeted MATα Disruption by loxP-kanMX-loxP Cassette and Construction of ΔMATα Mutants

Targeted deletion of Z. sapor expressed MATα was achieved by the integration of a loxP-kanMX-loxP cassette in ATCC 42981 genome.
1. PCR amplification of the disruption cassette holding a Kanamycin selectable marker targeted to host genome by homologous recombination.
2. Four ΔMATα ATCC 42981 mutants were constructed and confirmed by phenotypic analysis (growth on YPDA + G418) and PCR genotyping.

1.4 Cell-Identity Verification of ΔMATα Mutants

- MAT loci RT-PCR: as expected ΔMATα mutants transcribed MATα1 gene, but, surprisingly, they also actively transcribed MATα copy 2 genes. Why MATα is still expressed?
- Possible explanations:
  1. DIC1-MATα-SLA2 deletion could induce the linked CHA1, HMLα-SLA2 cassette de-silencing;
  2. HMLα transcription could not be completely repressed in the wild-type strain due to the recent acquisition of Sir1-driven silencing of HML/HMR transcription in Z. rouxii.
- HO RT-PCR: the deletion mutants constitutively transcribe the endonuclease. Nevertheless, HO expression itself does not assure that ATCC 42981 switches mating type.

2. ATCC 42981 and ABT 301’ Genome Assemblies

REDUNDANTS PIPELINE

- Genomes were assembled from illumina paired-end reads combining de novo assembly (dispSIM4), heterologous contigs reduction and scaffolding (Redundants).
- Two haplotypes A and B (~85% identity) were separated using Z. rouxii CBS 732 chromosomes as reference.
- Subsequently, contigs from both haplotypes were scaffolded independently based on synteny to reference chromosomes.
- Finally, the gaps were closed and both haplotypes were merged to give the full genome of each hybrid.

HAPLOTYPES DISSECTION

Genomes of Z. rouxii hybrids are composed of two haplotypes: one identical to CBS 372 (blue) and one, not yet identified, ~15% divergent (red). ATCC 42981 and ABT301 differ in chromosome composition suggesting an independent origin. The figure shows the sequencing coverage for all chromosomes of the three analyzed allotriploid strains. ATCC 42981 and CBS 4837 mostly share both haplotypes, while in ABT301 a decrease in read coverage (red arrows) suggests the lack of one chromosome from haplotype A (Chr: F in CBS 732) and part of ATCC 42981 scaffolds.

Sequence coverage

ATCC 42981
CBS 4837
ABT301’


We reconstructed how two independent CBS 732 stocks [namely CBS 732 R and CBS 732 P] underwent mating-type switching, generating a new MATα2 gene copy different from the silenced copy at the HMLα. Both the Z. rouxii cells are haploid but, differently from S. cerevisiae, they bypassed the cell-cycle control and expressed HO at the stationary phase. Despite this, mating-type switching occurred rarely or belatedly during Z. rouxii cell division. Despite being isogenic, the two cultures displayed distinct fertility response towards the opposite Z. rouxii mating testers.

Conclusions

ACTIVITY 1: HO expression does not assure mating-type switching; ii with one copy of MAT altered, ATCC 42981 does not behave as a haploid; iii) Z. sapor MATα deletion induces HMLα loci de-silencing or reveals the incomplete silencing of donor chromosomes in the wild-type strain.

ACTIVITY 2: ATCC 42981 genome assembly detected an additional MATα copy 2 cassette (table on the right). This could be co-expressed and explain why Z. sapor MATα disruption does not cause ATCC 42981 behaving as a haploid.

ACTIVITY 3: Mating-type switching of two independent CBS 732 stocks could be a plastic mechanism affecting genotypic instability and phenotypic novelties in haploid homothallic yeasts.

References