Whole Genome Sequencing, *de Novo* Assembly and Phenotypic Profiling for the New Budding Yeast Species *Saccharomyces jurei*

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**ABSTRACT** *Saccharomyces sensu stricto* complex consist of yeast species, which are not only important in the fermentation industry but are also model systems for genomic and ecological analysis. Here, we present the complete genome assemblies of *Saccharomyces jurei*, a newly discovered *Saccharomyces sensu stricto* species from high altitude oaks. Phylogenetic and phenotypic analysis revealed that *S. jurei* is more closely related to *S. mikatae*, than *S. cerevisiae*, and *S. paradoxus*. The karyotype of *S. jurei* presents two reciprocal chromosomal translocations between chromosome VI/VII and I/XIII when compared to the *S. cerevisiae* genome. Interestingly, while the rearrangement I/XIII is unique to *S. jurei*, the other is in common with *S. mikatae* strain IFO1815, suggesting shared evolutionary history of this species after the split between *S. cerevisiae* and *S. mikatae*. The number of Ty elements differed in the new species, with a higher number of Ty elements present in *S. jurei* than in *S. cerevisiae*. Phenotypically, the *S. jurei* strain NCYC 3962 has relatively higher fitness than the other strain NCYC 3947 under most of the environmental stress conditions tested and showed remarkably increased fitness in higher concentration of acetic acid compared to the other *sensu stricto* species. Both strains were found to be better adapted to lower temperatures compared to *S. cerevisiae*.

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*Sammar et al.* (2018) provide an in-depth analysis of the genetic and evolutionary relationships of *Saccharomyces sensu stricto* species. They highlight the importance of these species in both industrial applications and ecological contexts. The authors present complete genome assemblies for *S. jurei*, a new species discovered from high altitude oaks, and show that it shares a closer evolutionary relationship with *S. mikatae* than with *S. cerevisiae*. They report unique chromosomal translocations and differences in Ty element numbers between *S. jurei* and *S. cerevisiae*. Phenotypically, *S. jurei* has a higher fitness under various environmental stresses, particularly higher acetic acid tolerance, and is better adapted to lower temperatures. This study contributes to our understanding of the genetic, evolutionary, and ecological diversity of *Saccharomyces sensu stricto* species, which are crucial in the fermentation industry and as model systems for genomics and ecology.
speciation of yeast strains in nature. Genome variation provides the raw material for evolution, and may arise by various mechanisms including gene duplication, horizontal gene transfer, hybridization and micro and macro rearrangements (Fischer et al. 2001; Seoighe et al. 2000; Lynch 2002; Hall et al. 2005; Naseeb et al. 2017a; Naseeb et al. 2016; Naseeb and Delneri 2012). Synteny conservation studies have shown highly variable rates of genetic rearrangements between individual lineages both in vegetative and in yeasts (Bourque et al. 2005; Fischer et al. 2006; Vakirlis et al. 2016). This genome variation is a means of evolutionary adaptation to environmental changes. An understanding of the genetic machinery linked to phenotypic variation provides knowledge of the distribution of Saccharomyces species in different environments, and their ability to withstand specific conditions (Goddard and Greig 2015; Jouhten et al. 2016; Brice et al. 2018).

Recently, we isolated two strains (NCYC 3947T and NCYC 3962) of Saccharomyces jurei from Quercus robur bark and surrounding soil (Naseeb et al. 2017b). The initial sequencing of ITS1, D1/D2 and seven other nuclear genes showed that both strains of S. jurei were closely related to S. mikatae and S. paradoxus and grouped in Saccharomyces sensu stricto complex. We also showed that S. jurei can readily hybridize with other sensu stricto species but the resulting hybrids were sterile (Naseeb et al. 2017b). Here, we represent high quality de novo sequence and assembly of both strains (NCYC 3947T and NCYC 3962) of S. jurei. The phylogenetic analysis placed S. jurei in the sensu stricto clade, in a small monophyletic group with S. mikatae. By combining Illumina HiSeq and PacBio data, we were able to assemble full chromosomes and carry out synteny analysis. Moreover, we show that S. jurei NCYC 3962 had higher fitness compared to NCYC 3947T under different environmental conditions. Fitness of S. jurei strains at different temperatures showed that it was able to grow at wider range of temperatures (12°-37°).

**MATERIAL AND METHODS**

**Yeast strains**

Strains used in this study are presented in Table 1. All strains were grown and maintained on YPDA (1% w/v yeast extract, 2% w/v Bactopeptone, 2% v/v glucose and 2% w/v agar). Species names and strains number are stated in Table 1.

**DNA Extraction**

For Illumina HiSeq, the total DNA was extracted from an overnight grown culture of yeast strains by using the standard phenol/chloroform method described previously (Fujita and Hashimoto 2000) with some modifications. Briefly, 5 ml of overnight grown yeast cells were centrifuged and resuspended in 500 µl EB buffer (4M sorbitol, 500mM EDTA and 1M DTT) containing 1 mg/ml lyticase. The cells were incubated at 37° for 1 hr. Following incubation, the cells were mixed with stop solution (3M NaCl, 100mM Tris pH 7.5 and 20mM EDTA) and 60 µl of 10% SDS. The cell suspension was vortexed and mixed with 500 µl phenol-chloroform. The samples were centrifuged at 13000 rpm for 2 min to separate the aqueous phase from the organic phase. The upper aqueous phase was transferred to a clean 1.5 ml tube and phenol-chloroform step was repeated twice until a white interface was no longer present. The aqueous phase was washed with 1 ml absolute ethanol by centrifugation at 13000 rpm for 10 min. The pellet was air dried and resuspended in 30 µl of sterile milliQ water.

Genomic DNA for PacBio sequencing was extracted using Qiagen Genomic-tip 20/G kit (cat. No. 10223) following manufacturer’s recommended instructions. The yield of all DNA samples was assessed by the nanodrop spectrophotometer (ND-1000) and by Qubit 2.0 fluorometer (catalog no. Q32866). Purity and integrity of DNA was checked by electrophoresis on 0.8% (w/v) agarose gel and by calculating the A260/A280 ratios.

**Library preparation for Illumina and PacBio sequencing**

Paired end whole-genome sequencing was performed using the Illumina HiSeq platform. FastQC (Babraham Bioinformatics) was used to apply quality control to sequence reads, alignment of the reads was performed using BOWTIE2 (Langmead and Salzberg 2012) and post-processed using SAMTOOLs (Li et al. 2009).

For Pacbio sequencing, genomic DNA (10 µg) of NCYC 3947T and NCYC 3962 strains was first DNA damage repaired, sheared with Covaris G-tube, end repaired and exonuclease treated. SMRTbell library (10-20kb size) was prepared by ligation of hairpin adapters at both ends according to Pacbio recommended procedure (Pacific Biosciences, No: 100-259-100). The resulting library was then size selected using Blue Pippin with 7-10kb cut-off. Sequencing run was performed on PacBio RS II using P6/C4 chemistry for 4 hr. The genome was assembled using SMRT analysis and HGAP3 pipeline was made using default settings.

**Genome assembly, annotation, orthology and chromosomal structural plots**

The PacBio genome assemblies were assembled using hierarchical genome-assembly process (HGAP) (Chin et al. 2013). Protein coding gene models were predicted using Augustus (Stanke and Morgenstern 2005) and the Yeast Genome Annotation Pipeline (Byrne and Wolfe 2005). In addition, protein sequences from other Saccharomyces species were aligned to the genome assembly using tblastn (Gertz et al. 2006). These predictions and alignments were used to produce a final set of annotated genes with the Apollo annotation tool (Lewis et al. 2002). The protein sequences were functionally annotated using InterProScan.
Phenotypic assays

Temperature tolerance: Fitness of *S. jurei* strains and *Saccharomyces sensu stricto* type strains was examined using FLUOstar optima microplate reader at 12\(^\circ\), 16\(^\circ\), 20\(^\circ\), 25\(^\circ\), 30\(^\circ\) and 37\(^\circ\). Cells were grown from a starting optical density (OD) of 0.15 to stationary phase in YPD (1% w/v yeast extract, 2% w/v Bacto-peptone and 2% w/v glucose) medium. The growth OD\(_{595}\) was measured every 5 min with 1 min shaking for 72 hr. Growth parameters, lag phase (\(\lambda\)), maximum growth rate (\(\mu_{\max}\)), and maximum biomass (\(A_{\max}\)) were estimated using R shiny app on growth curve analysis (https://kobchai-shinyapps01.shinyapps.io/growth_curve_analysis/).

Environmental stress: Strains were screened for tolerance to environmental stressors using a high-throughput spot assay method. Cells were grown in a 96-well plate containing 100 \(\mu\)l YPD in four replicates at 30\(^\circ\) for 48 hr. The yeast strains grown in 96-well plate were sub-cultured to a 384 well plate to achieve 16 replicates of each strain and grown at 30\(^\circ\) for 48 hr. Singer ROTOR HDA robot (Singer Instruments, UK) was used to spot the strains on (i) YPDA + 0.4% & 0.6% acetic acid, (ii) YPDA+ 4mM & 6mM H\(_2\)O\(_2\), (iii) YPDA+ 2.5mM & 5mM CuSO\(_4\), (iv) YPDA+ 2% & 5% NaCl, (v) YPDA+ 5% & 10% Ethanol (vi) YPA+ 15% maltose and (vii) YPA+ 30% & 35% glucose. The spot assay plates were incubated at 30\(^\circ\) and high-resolution images of phenotypic plates were taken using phenobooth after 3 days of incubation (Singer Instruments, UK). The colony sizes were calculated in pixels using phenosuite software (Singer Instruments, UK). The high-quality de novo sequencing of *S. jurei* NCYC 3947\(^T\) was performed using Illumina HiSeq and Pacbio platforms. We obtained approximately 9.02 \(\times\) 10\(^5\) and 4.5 \(\times\) 10\(^5\) reads for NCYC 3947\(^T\) and NCYC 3962 respectively. We obtained 2 \(\times\) 101 bp reads derived from \(~200\) bp paired-end reads which were assembled in 12 Mb genome resulting in a total coverage of 250x based on high quality reads. The sequencing results and assembled contigs are summarized in Tables 2, 3, and 4. By combining the Illumina mate pair and Pacbio sequencing we were able to assemble full chromosomes of *S. jurei* NCYC 3947\(^T\) and NCYC 3962 (Tables 5 and 6). The total genome size (\(~12\) Mb) obtained for both strains of *S. jurei* was comparable to the previously published genomes of *Saccharomyces sensu stricto* species (Scannell et al. 2011; Goffeau et al. 1996; Liti et al. 2013; Baker et al. 2015).

S. jurei genome prediction and annotation

The high-quality *de novo* assembly of *S. jurei* NCYC 3947\(^T\) genome resulted in 5,794 predicted protein-coding genes for *S. jurei*, which is

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**Table 2: Summary of *S. jurei* NCYC 3947\(^T\) genome sequencing and assembly using Hi-seq platform**

| Metric          | Contigs | Contigs >= 500bp | Scaffolds | Scaffolds >= 500bp |
|-----------------|---------|------------------|-----------|--------------------|
| Number          | 810     | 250              | 753       | 211                |
| Total Length    | 11,938,007 | 11,869,594   | 11,940,421 | 11,869,594         |
| Length Range    | 87-673,524 | 525-673,524   | 87-673,524 | 525-673,524        |
| Average Length  | 14,738  | 56,254           | 15,857    | 56,254             |
| N50             | 172,207 | 279,631          | 279,631   |                    |

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**Table 3: Summary of *S. jurei* NCYC 3962 genome sequencing and assembly using Hi-seq platform**

| Metric          | Contigs | Contigs >= 500bp | Scaffolds | Scaffolds >= 500bp |
|-----------------|---------|------------------|-----------|--------------------|
| Number          | 3719    | 987              | 3618      | 933                |
| Total length    | 11,760,925 | 11,419,281   | 11,768,034 | 11,441,494         |
| Length range    | 59-80,684 | 507-80,684   | 59-80,684 | 507-80,684         |
| Average length  | 3,162   | 11,569           | 3,252     | 12,263             |
| N50             | 20,806  | 21,318           | 21,928    | 22,552             |

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@Jones et al. 2014. Orthologous relationships with *S. cerevisiae* S288C sequences were calculated using InParanoid (Berglund et al. 2008). Non-coding RNAs were annotated by searching the RFAM database (Nawrocki et al. 2015) using Infernal (Nawrocki and Eddy 1997). Further tRNA predictions were produced using tRNAscan (Lowe and Eddy 1997). Repeat sequences were identified in Repbase (Bao et al. 2015) using Repeat Maser (Smit et al. 2013–2015). The dotplots were constructed by aligning *S. jurei* genome to the *S. cerevisiae* S288C genome using NCURmer and plotted using MUMmerplot (Kurtz et al. 2004). These features are available to browse via a UCSC genome browser (Kent et al. 2002) track hub (Raney et al. 2014). Single nucleotide polymorphisms (SNPs) were identified using Atlas-SNP2 (Challis et al. 2012).
similar to the published genomes of other sensu stricto species (Baker et al. 2015; Liti et al. 2009; Liti et al. 2013; Scannell et al. 2011; Walther et al. 2014). Of the predicted protein-coding genes, 5,124 were shown to have many to many relationship (multiple S. cerevisiae genes in paralogous cluster with multiple S. jurei genes (Table S2), 31 genes were in many to one relationship (many genes in S. cerevisiae are in an paralogous cluster with a single S. jurei gene; most of these were found to be retrotransposons; Table S3) and 50 genes were in one to many relationships (one S. cerevisiae gene in an paralogous cluster with many S. jurei genes; Table S4). Interestingly, we found an increase in the copy number of maltose metabolism and transport genes (IMA1, IMA5, MAL31, and YPR196W-2 copies of each gene), flocculation related gene (FLO1-2 copies) and hexose transporter (HXT8-3 copies). Increased dosage of these genes in S. jurei could have conferred selective advantage toward better sugar utilization (Lin and Li 2011; Ozcan and Johnston 1999; Soares 2011; Adamczyk et al. 2016). Genes encoding for PAU proteins (a member of the seripauperin multigene family), copper resistance and salt tolerance related genes were found to be present in fewer copies in S. jurei genome compared to S. cerevisiae. This variation in copy number of genes in a genome can have phenotypic and physiological effects on the species (Landry et al. 2006; Adamo et al. 2012; Gorter de Vries et al. 2017).

We also searched for the presence of repetitive elements in S. jurei NCYC 3947$^T$ and NCYC 3962 using BLAST and compared them to the Ty elements in S. cerevisiae. We detected Ty1-LTR, Ty2-LTR, Ty2-I-int, Ty3-LTR, Ty3-I and Ty4 sequences in both strains of S. jurei. Interestingly, we found an increased number of Ty1-LTR, Ty2-LTR, Ty3-LTR and Ty4 elements in S. jurei genome compared to S. cerevisiae (Table 7). High copy numbers of Ty1, Ty2, and Ty3 transposable elements have also been reported in different strains of S. cerevisiae, e.g., Ty1 and Ty2 in French Dairy, Ty1 in Alpechín, Ty1 in Mexican Agave, and Ty3 in Ecuadorian clade (Peter et al. 2018; Bleykasten-Grosshans et al. 2013). Repetitive sequences are found in genomes of all eukaryotes and can be a potential source of genomic instability since they can recombine and cause chromosomal rearrangements, such as translocations, inversions and deletions (Naseeb et al. 2016; Shibata et al. 2009; Chan and Kolodner 2011).

**Saccharomyces jurei share a chromosomal translocation**

*With Saccharomyces mikatae IFO 1815*

To check the presence or absence of genomic rearrangements in S. jurei, we compared the chromosome structures between S. jurei NCYC 3947$^T$ and S. jurei NCYC 3962 (Figure 1A), between S. cerevisiae S288C and S. mikatae IFO1815 (Figure 1B), between S. jurei NCYC 3947$^T$ and S. cerevisiae S288C (Figure 2A) and between S. jurei NCYC 3947$^T$ and S. mikatae IFO1815 (Figure 2B). The two S. jurei strains had a syntenic genome (Figure 1A), while we identified two chromosomal translocations with S. cerevisiae S288C (Figure 2A). One translocation is unique to S. jurei and is located between chromosomes I and XIII (Figure 2, red ovals), while the second translocation is located between chromosomes VI and VII in the same position of the previously identified translocation in S. mikatae IFO1815 (Figure 2, black ovals).

The breakpoints of the translocation I/XIII are in the intergenic regions between uncharacterized genes. The breakpoints neighborhood is surrounded by several Ty elements (Ty1-LTR, Ty4, and Ty2-LTR) and one rRNA, which may have caused the rearrangement (Bridier-Nahmias et al. 2015; Fischer et al. 2000; Liti et al. 2013; Mieczkowska et al. 2006). The translocation in common with S. mikatae shares the same breakpoints between open reading frames (ORFs) YFR006W and YFR009W on chromosome VI, and between ORFs YGR021W and YGR026W on chromosome VII. This translocation is also shared by both strains of S. mikatae, but not with other Saccharomyces sensu stricto species. Overall this suggests a common evolutionary history between these strains and species, however an adaptive value of this rearrangement.

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**Table 4 Summary of S. jurei NCYC 3947$^T$ and NCYC 3962 genome assembly using PacBio platform**

| Metric                  | S. jurei NCYC 3947 | S. jurei NCYC 3962 |
|-------------------------|--------------------|--------------------|
| Contigs                  | 35                 | 57                 |
| Max contig length        | 1,474,466          | 1,470,125          |
| Contig N50               | 738,741            | 652,030            |
| Total assembly size      | 12,306,756         | 12,932,708         |

**Table 5 Total lengths of chromosomes assembled in S. jurei NCYC 3947$^T$**

| Sequence name             | Length (bp) including gaps |
|---------------------------|----------------------------|
| chrL_1_chrXIII.2          | 809,572                    |
| chrII                     | 809,280                    |
| chrIII                    | 308,350                    |
| chrV                      | 1,474,466                  |
| chrV                      | 584,553                    |
| chrVI.1_chrVII.2          | 730,011                    |
| chrVI.2_chrVIII.1         | 638,210                    |
| chrVIII                   | 534,462                    |
| chrX                      | 434,517                    |
| chrXI                     | 738,741                    |
| chrXII.1                  | 458,950                    |
| chrXII.2                  | 568,540                    |
| chrL2_chrXIII.1           | 334,136                    |
| chrXIV                    | 749,072                    |
| chrXV                     | 1,068,672                  |
| chrXVI                    | 920,427                    |
| chrMT                     | 105,732                    |

**Table 6 Total lengths of chromosomes assembled in S. jurei NCYC 3962**

| Sequence name             | Length (bp) including gaps |
|---------------------------|----------------------------|
| chrL_1_chrXIII.2          | 756,315                    |
| chrII                     | 814,183                    |
| chrIII                    | 329,028                    |
| chrIV                     | 1,470,125                  |
| chrV                      | 570,437                    |
| chrVI.1_chrVII.2          | 723,619                    |
| chrVI.2_chrVIII.1         | 652,030                    |
| chrVIII                   | 536,516                    |
| chrX                      | 439,662                    |
| chrX1                     | 487,336                    |
| chrX2                     | 258,684                    |
| chrX11                    | 676,065                    |
| chrXII.1                  | 475,978                    |
| chrXII.2                  | 571,082                    |
| chrL2_chrXIII.1           | 334,998                    |
| chrXIV                    | 790,124                    |
| chrXV                     | 474,048                    |
| chrXVI.2                  | 240,703                    |
| chrXV.3                   | 236,823                    |
| chrXVII                   | 114,889                    |
| chrXVI                    | 806,586                    |
| chrMT                     | 110,829                    |
or a case of breakpoint re-usage cannot be ruled out since rearrangements can be adaptive with evidence both from nature and lab setting. (Chang et al. 2013; Dunham et al. 2002; Avelar et al. 2013; Colson et al. 2004; Adams et al. 1992; Fraser et al. 2005; Hewitt et al. 2014). Several natural isolates of *S. cerevisiae* present karyotypic changes (Hou et al. 2014) and the reciprocal translocation present between chromosomes VIII and XVI is able to confer sulphite resistance to the yeasts strains in vineyards (Perez-Ortin et al. 2002). Furthermore, lab experimental evolution studies in different strains of *S. cerevisiae* when evolved under similar condition end up sharing the same breakpoints (Dunham et al. 2002). Previous studies on mammalian systems have shown that breakpoints maybe reused throughout evolution at variable rates (Larkin et al. 2009; Murphy et al. 2005), and breakpoint re-usage has also been found between different strains of *S. pastorianus* (Hewitt et al. 2014).

### Novel genes present in *S. jurei*

The comparison between *S. jurei* and *S. cerevisiae* genome showed 622 differentially present genes. 179 open reading frames (ORFs) were predicted to be novel in *S. jurei* when compared to *S. cerevisiae* reference S288C strain (Table S5). To further confirm if these ORFs were truly novel, we analyzed the sequences in NCBI nucleotide database and in *Saccharomyces* Genome Database (SGD) against all the fungal species. We found 4 novel ORFs that have no significant match to any of the available genomes (Table S5-shown in red). 5 ORFs gave partial similarity to different fungal species such as *Vanderwaltozyma polyspora*, *Kluyveromyces marxianus*, *Torulaspora delbrueckii*, *Zygosaccharomyces rouxii*, *Hyphopichia burtonii*, *Kazachstania africana*, *Trichocera brevicornis*, *Lachancea walti*, and *Naumovozyma castellii* (Table S5-yellow highlighted). Majority of the remaining sequences gave full or partial matches to *S. cerevisiae*.

### Table 7 Counts of Ty elements in *S. cerevisiae*, *S. jurei* NCYC 3947^T^ and NCYC 3962

| Ty elements | Ty elements annotation | Counts in *S. cerevisiae* | Counts in *S. jurei* NCYC 3947^T^ | Counts in *S. jurei* NCYC 3962 |
|-------------|------------------------|---------------------------|-----------------------------------|---------------------------------|
| Ty          | Yeast Ty transposable element | 164                       | 71                                | 74                              |
| Ty1-LTR     | Ty1 LTR-retrotransposon from yeast (LTR) | 124                       | 276                               | 272                             |
| Ty2-LTR     | Ty2 LTR-retrotransposon from yeast (LTR) | 108                       | 118                               | 117                             |
| Ty2-I-int   | Ty2 LTR-retrotransposon from yeast (internal portion) | 15                        | 2                                 | 2                               |
| Ty3-LTR     | *S. paradoxus* Ty3-like retrotransposon, Long terminal repeat | 61                        | 70                                | 71                              |
| Ty3-I       | *S. paradoxus* Ty3-like retrotransposon, Internal region. | 2                         | 1                                 | 1                               |
| Ty4         | Gag homolog, Ty4B = protease, integrase, reverse transcriptase, and RNase H domain containing protein (retrotransposon Ty4) | 51                        | 164                               | 162                             |

![A] S. jurei NCYC 3947^T^ vs. S. jurei NCYC 3962

![B] S. cerevisiae vs S. mikatae IFO1815

*Figure 1* Dot plot alignments comparing the chromosome sequence identity of *S. jurei* NCYC 3947^T^ vs. *S. jurei* NCYC 3962 (A) and *S. cerevisiae* S288C vs. *S. mikatae* IFO1815 (B). The broken lines represent chromosomal translocations between chromosomes VI / VII and XVI / VII.
natural isolates (Strope et al. 2015; Peter et al. 2018), S. paradoxus, S. mikatae, S. kudriavzevii, S. bayanus, S. uvarum, and S. eubayanus.

Moreover, we also found 462 ORFs, which are present in S. cerevisiae genome but were lost in S. jurei (Table S6). The Gene Ontology (GO) analysis of these genes showed significant enrichment of RNA-directed DNA polymerase activity, aryl-alcohol dehydrogenase (NAD+) activity, DNA-directed DNA polymerase activity, and asparaginase activity. The majority of genes which were novel or lost in S. jurei were found to be subtelomeric or telomeric, in regions known to show higher genetic variations (Bergström et al. 2014).

The genes lost in S. jurei encompass functionally verified ORFs, putative genes and uncharacterized genes. Some of the verified ORFs included ribosomal subunits genes, asparagine catabolism genes, alcohol dehydrogenase genes, hexose transporters, genes involved in providing resistance to arsenic compounds, phosphopyruvate hydratase genes, iron transport facilitators, ferric reductase genes and flocculation related genes.

We found that S. jurei genome lacks four out of seven alcohol dehydrogenase (AAD) genes including the functional AAD4 gene, which is involved in oxidative stress response (Delneri et al. 1999a; Delneri et al. 1999b). Although S. jurei has lost AAD4 gene, however, it was able to tolerate oxidative stress caused by 4mM H2O2 (Figure 3A).

All four genes of the ASP3 gene cluster located on chromosome XII are absent in S. jurei. It was not surprising since this gene cluster is only known to be present in S. cerevisiae strains isolated from industrial and laboratory environments and lost from 128 diverse fungal species (Gordon et al. 2009; League et al. 2012). These genes are up-regulated during nitrogen starvation allowing the cells to grow by utilizing extracellular asparagine as a nitrogen source.

The hexose transporter family consists of 20 putative HXT genes (HXT1-HXT17, GAL2, SNF3, and RGT2) located on different chromosomes (Boles and Hollen 1997; Kruckeberg 1996) of which HXT15, HXT16 and HXT2 are absent from S. jurei. Under normal conditions, only 6 HXT genes (HXT1 and HXT3-HXT7) are known to play role in glucose uptake suggesting that loss of 3 HXT genes from S. jurei is unlikely to affect glucose transport (Lin and Li 2011).

**Heterozygosity and strain divergence in the S. jurei**

To detect genetic divergence between the two strains we mapped SNPs between the strains (NCYC 3947T vs. S. cerevisiae 3962), while to detect heterozygosity, we mapped the Single Nucleotide Polymorphisms (SNPs) in the two sets of alleles within the novel strains (NCYC 3947T vs. NCYC 3947T, and NCYC 3962 vs. NCYC 3962). We found 6227 SNPs between the two strains, showing a genetic divergence between them, which is relatively lower compared to the genetic divergence found among S. cerevisiae strains. Moreover, 278 and 245 SNPs were found within NCYC 3947T and NCYC 3962 strains respectively, indicating a low level of heterozygosity within each strain (Table 8). 139 SNPs were found to be common to both strains. Previous studies on S. cerevisiae and S. paradoxus strains from different lineages have shown that the level of heterozygosity is variable, with a large number of strains showing high level of heterozygosity isolated from human associated environments (Magwene et al. 2011; Tsai et al. 2008). A more recent study on 1011 S. cerevisiae natural strains showed that 63% of the sequenced isolates were heterozygous (Peter et al. 2018).

**Phylogenetic analysis**

A first phylogeny construction using ITS/D1+D2 sequence analysis showed that S. jurei is placed in the tree close to S. cerevisiae, S. mikatae and S. paradoxus (Naseeb et al. 2017b). Here, we reconstructed the phylogeny using a multigene concatenation approach, which combines many genes together giving a large alignment (Fitzpatrick et al. 2006; Brown et al. 2001; Baldauf et al. 2000). Combination of concatenated genes improves the phylogenetic accuracy and helps to resolve the nodes and basal branching (Rokas et al. 2003). To reconstruct the evolutionary events, we concatenated 101 universally distributed orthologs obtained from complete genome sequencing data (Table S7). Both novel strains were located in one single monophyletic group, with
the S. mikatae (Figure 4). Since S. jurei also have a chromosomal translocation in common with S. mikatae, it further shows that the two species share similar evolutionary history and hence present in the same group on the phylogenetic tree.

**Introgression analysis**

To determine whether the two S. jurei strains possessed any introgressed region from other yeast species, we compared S. jurei genome with those of S. cerevisiae, S. mikatae, S. paradoxus and S. kudriavzevii. We did not observe introgression of any full-length genes or large segments of the genome (>1000 bp) in S. jurei. However, in both novel strains, we identified seven small DNA fragments (300 bp-700 bp) belonging to five different genes, which may have derived from S. paradoxus or S. mikatae (Table S8). DNA fragments from all the genes (CSS3, IMA5, MAL33, YAL003W) with the exception of YDR541C, showed a high sequence similarity to S. paradoxus genome, indicating putative introgression from S. paradoxus to S. jurei (Table S8).

Introgression of genetic material can easily occur in Saccharomyces species by crossing the isolates to make intraspecific or interspecific hybrids (Fischer et al. 2000; Naumov et al. 2000). Among the Saccharomyces sensu stricto group, introgressions have been demonstrated in natural and clinical yeast isolates (Liti et al. 2006; Zhang et al. 2010; Wei et al. 2007; Muller and McCusker 2002; Usher and Bond 2009; Dunn et al. 2012). It is generally believed that introgressed regions are retained, as they may be evolutionarily advantageous (Strope et al. 2015; Novo et al. 2009). Previous studies have demonstrated that introgression in S. cerevisiae is relatively common and a majority of the genes are derived from introgression with S. paradoxus (Strope et al. 2015; Warringer et al. 2011; Novo et al. 2009; Liti et al. 2006; Peter et al. 2018).

**Phenotypic profiling of S. jurei**

We performed large-scale phenotypic profiling under various stress conditions and at different temperatures to capture the fitness landscape of S. jurei (strains NCYC 3947T and NCYC 3962) relative to other Saccharomyces sensu stricto species. Type strains of all Saccharomyces sensu stricto species were used for fitness analysis. Colony size was taken as a proxy for fitness score (see methods). Generally the fitness of S. jurei NCYC 3962 in different environmental stressor conditions was higher compared to S. jurei NCYC 3947T (Figure 3). Remarkably, only S. jurei NCYC 3962 was able to grow well on higher concentrations of acetic acid (Figure 3). Like most of the other Saccharomyces yeast species, both strains of S. jurei can also grow in media containing 10% ethanol. Although S. eubayanus showed the highest fitness in media containing 15% maltose, both strains of S. jurei were also able to tolerate high concentrations of maltose. Moreover, S. jurei NCYC 3962 was able to better tolerate higher concentrations of H2O2, CuSO4 and NaCl compared to most of the other sensu stricto species (Figure 3).

Saccharomyces yeast species can acquire copper tolerance either due to an increase in CUP1 copy number (Warringer et al. 2011) or due to the use of copper sulfate as a fungicide in vineyards (Fay et al. 2004; Perez-Ortin et al. 2002). The genomic analysis shows that both strains of S. jurei possess one copy of CUP1, indicating other factors maybe associated with copper tolerance.

Phenotypically, both strains of S. jurei clustered with S. mikatae and S. paradoxus, which is in accordance with our phylogenetic results, and, interestingly, the brewing yeast S. eubayanus was also present in the same cluster (Figure 3). This may indicate that in spite of the phylogenetic distance, S. eubayanus may have shared similar ecological conditions with the other above mentioned species.

We also evaluated the fitness of S. jurei strains in comparison to Saccharomyces sensu stricto species at different temperatures, taking

![Figure 3](https://example.com/image3.png)

**Table 8** Approximate numbers of SNPs in S. jurei NCYC 3947T and NCYC 3962 genome

| Reference genome | Genome mapped | SNPs |
|------------------|---------------|------|
| NCYC 3947T       | NCYC 3947T    | 278  |
| NCYC 3962        | NCYC 3962     | 245  |
| NCYC 3947T       | NCYC 3947T    | 5702 |
| NCYC 3962        | NCYC 3947T    | 6227 |
into account growth parameters such as lag phase ($\lambda$), maximum growth rate ($\mu_{\text{max}}$), and maximum biomass ($A_{\text{max}}$) (Tables S9-S11). The optimum growth of NCYC 3947 and NCYC 3962 was at 25° and 30° respectively (Table S10). Both strains of $S. jurei$ are able to grow at a high temperatures (i.e., 37°) compared to $S. kudriavzevii$, $S. pastorianus$, $S. arboricola$, $S. uvarum$, and $S. eubayanus$, which are unable to grow at 37° (Table S10). The ability of $S. jurei$ strains to grow well both at cold and warm suggest that this species evolved to be a generalist rather than a specialist in terms of thermopreference. The growth profiles captured at different temperatures for the other Saccharomyces species was in accordance to the previously published study (Salvadó et al. 2011).

**Conclusions**

High quality de novo assembly of two novel strains of $S. jurei$ (NCYC 3947 and NCYC 3962) has been carried out using short and long reads sequencing strategies. We obtained a 12 Mb genome and were able to assemble full chromosomes of both strains. We found two reciprocal chromosomal translocations in $S. jurei$ genome, between chromosomes I/XIII and VI/VII. The translocation between chromosomes I/XIII is unique to $S. jurei$ genome, whereas the translocation between VI/VII is shared with $S. mikatae$ IFO1815 and IFO1816. This suggests a common origin between $S. jurei$ and $S. mikatae$ and $S. jurei$ evolved after acquiring the translocation between chromosomes I/XIII, while $S. mikatae$ 1815 acquired a second translocation between chromosomes XVI/VII. Moreover, both strains of $S. jurei$ showed low heterozygosity within themselves and were genetically diverged possessing 6227 SNPs between them. We found 4 novel ORFs that had no significant match to any of the available genomes. $S. jurei$ genome had an increased number of Ty elements compared to $S. cerevisiae$ and showed no signatures of introgression. The phylogenetic analysis showed that the novel species is closely related to $S. mikatae$, forming a single monophyletic group.

Phenotypically, the environmental stressor profiles of $S. jurei$ are similar to those of with $S. mikatae$, $S. paradoxus$, $S. cerevisiae$ (which further reiterate that $S. jurei$ is closely related to these species) and $S. eubayanus$. We found that $S. jurei$ NCYC 3962 compared to other sensu stricto species was able to grow well at high concentrations of acetic acid. In general, $S. jurei$ NCYC 3962 showed relatively higher fitness compared to $S. jurei$ NCYC 3947 under most of the environmental stress conditions tested. Both strains of $S. jurei$ showed similar growth rate at a relatively low temperature, however, NCYC 3962 showed increased fitness compared to NCYC 3947 at higher temperatures. The sequencing data and the large-scale phenotypic screening of this new species provide the basis for future investigations of biotechnological and industrial importance.

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