Genomic Exploration of the Hemiascomycetous Yeasts: 7. *Saccharomyces servazzii*

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Abstract The genome of *Saccharomyces servazzii* was analyzed with 2570 random sequence tags totalling 2.3 Mb. BLASTX comparisons revealed a minimum of 1420 putative open reading frames with significant homology to *Saccharomyces cerevisiae* (58% aa identity on average), two with *Schizosaccharomyces pombe* and one with a human protein, confirming that *S. servazzii* is closely related to *S. cerevisiae*. About 25% of the *S. servazzii* genes were identified, assuming that the gene complement is identical in both yeasts. *S. servazzii* carries very few transposable elements related to Ty elements in *S. cerevisiae*. Most of the mitochondrial genes were identified in eight contigs altogether spanning 25 kb for a predicted size of 29 kb. A significant match with the *Kluyveromyces lactis* linear DNA plasmid pGKL-1 encoded RF4 killer protein suggests that a related plasmid exists in *S. servazzii*. The sequences have been deposited with EMBL under the accession numbers AL402279–AL404848.

Key words: Spliceosomal intron; Plasmid; Mitochondrial DNA; *Saccharomyces* sensu lato

1. Introduction

Saccharomyces servazzii belongs to the *Saccharomyces* sensu lato group. Eight different isolates, obtained from soil, feces and tree exudates, are available in various collections. *S. servazzii*, like the other *Saccharomyces* species, is homothallic. However, it differs significantly from the other species of the genus, except *S. unisporus*, as it has limited assimilation and fermentation of carbon sources. It only ferments glucose and galactose. In addition to these sugars, this yeast is able to assimilate trehalose and glycerol. It further differs from *S. unisporus* in its inability to utilize cadaverin, ethylamine and L-lysine as sole nitrogen sources. *S. servazzii* can grow naturally on a high cycloheximide concentration (0.1%) [1]. Some non-conventional yeasts, like *Schwanniomyces occidentalis* and *Kluyveromyces lactis*, share this antibiotic resistance property conferred by a single ribosomal protein, L41. *S. cerevisiae*, like most other yeasts, is naturally sensitive to cycloheximide and resistance to this antibiotic is due to mutations in ribosomal genes.

Distinction between *S. servazzii* and other *Saccharomyces* sensu lato species (*S. castelli*, *S. dairenensis*, *S. exiguus* and *S. unisporus*) was first obtained through spectrophotometric DNA reassociation studies [2]. The phylogenetic position of *S. servazzii* is quite unusual. On the basis of 26S ribosomal DNA (rDNA) comparison, it was proposed that this species could form, together with *S. unisporus* to which it is closely related, a phylogenetic group distinct from the other *Saccharomyces* and the non-*Saccharomyces* [3]. This was confirmed by studies on the complete 18S rDNA comparison performed on all *Saccharomyces* species including close species from different genera [4].

The molecular biology of this yeast is not developed. Sequences in databases represent mainly ribosomal rRNA genes and rDNA intergenic sequences that were used for phylogenetic studies. The sequence of a mitochondrial gene, *ATP9*, and partial sequences of mitochondrial 15S and 21S rRNA genes are also available. A recent study on the evolution of mitochondrial subunit *COX2* and *COX3* specific activators led to the cloning of homologues of *S. cerevisiae PET111, PET122, COX7* and *OXA1*. Interestingly, *S. servazzii PET111* and *PET122* do not complement mutations in the corresponding genes of *S. cerevisiae*, indicating that gene sequences have strongly diverged [5].

Like most *Saccharomyces* sensu lato species tested, *S. servazzii* was moderately inducible for the petite phenotype with ethidium bromide [6]. Among the *Saccharomyces* sensu lato species, the sizes of the mitochondrial genomes fall into two classes: one at around 50 kb and one between 25 and 30 kb. *S. servazzii* has an estimated mitochondrial genome size of 29 kb [6].

Earlier electrophoretic karyotypes of *S. servazzii* revealed seven chromosomal bands varying between 700 and 1600 kb, leading to an estimated genome size of 10.8 Mb [7]. A recent study showed at least 12 bands (570–1950 kb) for an estimated genome size of 12.3 Mb [8]. Similar patterns were obtained with *S. unisporus* isolates, consistent with the fact that these species are closely related [9]. Several markers isolated from *S. servazzii* chromosome 9 were shown to hybridize to different chromosomes of *S. cerevisiae* [10], revealing extensive rearrangements between chromosomes of these two yeasts.

2. Materials and methods

2.1. Yeast strain

The *S. servazzii* type strain CBS4311, a wild diploid isolate from soil (Finland), was used.

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2.2. S. servazzii genomic DNA library

S. servazzii was grown overnight at 28°C in 10 ml YPD (1% yeast extract, 1% peptone, 1% glucose). 2.5 μg of genomic DNA, prepared according to [11], was digested with 5 units of *Cvi*JI (CHIMERx, USA) at 37°C. Restriction fragments of 3–5 kb were size selected on a 1% agarose gel and purified using GeneClean II kit (Bio 101, USA). The pBAM3 vector [12] was digested with *Sma*I (Gibco BRL, USA) and dephosphorylated using alkaline phosphatase (Boehringer Mannheim, Germany). Vector (8 ng) and fragments (10–50 ng) were incubated overnight at 4°C with T4 DNA ligase (Gibco BRL, USA). *Escherichia coli* DH10B was transformed with the ligation mixture by electroporation (100 Ω, 25 μF, 1700 V/cm). Transformants were selected on LB medium containing ampicillin (100 μg/ml), IPTG (25 μg/ml) and X-gal (40 μg/ml). Plasmids from 96 white colonies were purified and digested with *Mlu*I to determine the presence and the size of the inserts. White colonies were then randomly picked, selected, grown in LB with ampicillin and glycerol (15%) and distributed in triplicate to 96 well plates subsequently stored at −80°C. The library consists of 1632 clones.

2.3. Properties of the DNA library and contigs

A total number of 2570 random sequence tags (RSTs) (average size of 898 bp) were sequenced [12]. Overall, 1178 inserts were sequenced at both ends. Only 214 clones had only one RST sequenced. The average size of the inserts from the library was found to be 3.72 kb (standard deviation, 0.62 kb). Five clones had overlapping RSTs.

Assembly of the contigs was performed as described in [13,14]. A total of 1296 RSTs were included in 295 contigs. A large majority of the contigs (87%) was composed of two (274) or three RSTs (83). Contig size varied from 0.15 to 6.1 kb. The contigs were subsequently used to define repeated sequences within the nuclear genome and extrachromosomal sequences. Annotations were performed as described in [14].

3. Results and discussion

3.1. Ribosomal DNA

Contig 414 (6133 bp) composed of 39 RSTs matches ribosomal DNA sequences. We identified the 18S gene (1–1773 bp), the 5.8S gene (2030–2182 bp) and the 26S gene (2431–5826 bp). Contig 414 lacks the first 39 bp at the 5′ end of the 18S gene [4]. The non-transcribed spacer is also truncated and no sequence homology to 5S could be found in contig 414. Only one single RST (AT0AA011C06T1) showed 97% identity over 107 bp with the *S. cerevisiae* 5S gene.

3.2. Retrotransposons

A total of 52 transposable elements were found in the *S. cerevisiae* sequenced strain [15]. Surprisingly, only four RSTs showed a significant match with *S. cerevisiae* Ty open reading frames (ORFs): one to TY1B (expected value 6×10^{-24}), one to TY3A (expected value 1×10^{-6}), and two to TY3B (expected value 5×10^{-9} and 2×10^{-7}). This indicates that few transposons are present and that Ty-like sequences are poorly conserved between *S. servazzii* and *S. cerevisiae*. A search for long terminal repeats was unsuccessful. This might indicate either that transposable elements are scarce in *S. servazzii* or that this species harbors different types of retrotransposons.

3.3. Plasmid

A significant match of the 3648 bp contig 409 (composed of 21 RSTs) to the RF4 protein encoded by the linear plasmid pGKL-1 of *K. lactis* [16,17] was found with 24% identity over the entire *K. lactis* protein (428 aa in length). A weaker match (32% over 162 aa) was also found with the YKP2 protein of the other *K. lactis* linear plasmid, pGKL-2. This suggests that *S. servazzii* harbors at least a linear DNA plasmid sharing some identity with one from *K. lactis*. A previous survey of a large number of the CBS yeast strains did not detect linear plasmids in *S. servazzii* ([18]; H. Fukuhara, personal communication). As pointed out by the author this could be due to limitations in the DNA extraction procedure, because a similar procedure revealed the existence of a linear DNA plasmid in another *Saccharomyces* sensu lato species, *S. kluyveri*.

3.4. Mitochondrial DNA

We identified eight contigs made up of 7–38 RSTs (total 161 RSTs) with sizes ranging from 2022 to 4947 bp representing part of the mitochondrial DNA. The genes *COX1*, *COX2*, *COX3* (cytochrome *c* oxidase subunits), *COB* (cytochrome *b*), *ATP6* and *ATP9* (ATP synthase subunits 6 and 9) were unambiguously identified. In contig 408, we found two ORFs corresponding to two *COB* exons separated by an intron that carried a putative intronic ORF homologous to maturase *Scb*I3 found in the third intron of the *S. cerevisiae COB* gene.

Four exons corresponding to *COX1* are carried by contig 413 but no significant matches with *S. cerevisiae* intronic ORFs were found. The 5′ end of the first *COX1* exon is truncated in contig 413. The first 24 amino acids of *COX1* were found at the 3′ end of contig 407.

The mitochondrial 21S rRNA and 15S rRNA genes were identified. No intron was found in the 21S rRNA gene. A total of 18 tRNA genes, including two tRNA_ser, were found to be distributed among all contigs, though some of these appeared in clusters, such as those in contigs 494 and 406, with clusters of five or six tRNA genes, respectively. We detected a strong sequence homology to the tRNA_his from *S. cerevisiae*, with an internal region of 20 bp that completely lacks homology. As this latter region probably carries the anticodon, the sequence cannot be unambiguously assigned to a tRNA_his, because it remains open (i) whether this extra sequence might correspond to an intron and (ii) which triplet within this might represent the 'real' anticodon. Finally, we found that all genes within each contig have the same orientation except for the homologue of the tRNA_thr anti that is located on the other strand as in *S. cerevisiae*. It is noticeable that, as in *S. cerevisiae*, the NADH dehydrogenase complex 1 is not present in *S. servazzii*. In our search, we found a single RST with a match (25% identity over 127 amino acids) with a subunit of the human NADH dehydrogenase complex that is involved in detoxification and biosynthesis but is not encoded mitochondrially [19].

The total content of the eight mitochondrial contigs covers more than 25 kb of the estimated size of 29 kb [6], indicating that the *S. servazzii* mitochondrial chromosome sequence is close to completion. The mitochondrial (G+C) content is 24% compared to a nuclear (G+C) content of 34%.

3.5. Identification of nuclear genes

Each of the RSTs, except those corresponding to rDNA and mtDNA, were compared to the complete non-redundant database of *S. cerevisiae* protein sequences [14]. The number of matches does not reflect the number of genes identified, since a large number of RSTs were overlapping and used to build the contigs. To estimate the number of *S. servazzii* ORFs with significant sequence similarity to *S. cerevisiae* ORFs, we checked whether RSTs with the same *S. cerevisiae* ORF overlapped over the homology segment or not. If they belonged to the same contig, they were assigned to a single *S. cerevisiae* orthologue. If two or more non-contigated RSTs

Table 1
List of identified *S. servazzii* paralogues

S. cerevisiae ORF	*S. servazzii* occurrence	*S. cerevisiae* gene family	*S. cerevisiae* gene name	*S. cerevisiae* function
YBL019w	2	Singleton	*ETH1*	exonuclease III homolog
YBR035c	2	Singleton	*PDX3*	pyridoxamine phosphate oxidase
YBR036c	2	Singleton	*CSG2*	calcium dependent regulatory protein
YER007w	3	Singleton	*PAC2*	involved in the stabilization of microtubules
YFL007w	2	Singleton	–	weak similarity to Mms19p
YFL047w	2	Singleton	–	similarity to *S. pombe* hypothetical protein SPAC2F7.18c
YGL246c	2	Singleton	–	weak similarity to *C. elegans* Dom-3 protein
YHR063c	2	Singleton	–	weak similarity to translational activator CBS2
YHR182w	2	Singleton	–	hypothetical protein
YLL004w	2	Singleton	*ORC3*	origin recognition complex, 62 kDa subunit
YOL070c	2	Singleton	–	hypothetical protein
YPL169c	2	Singleton	*MEX67*	factor for nuclear mRNA export
YAL023c	2	P7.2.f7.1	*PMT2*	mannosyltransferase
YBR068c	2	P23.1.f18.1	*BAP2*	leucine permease, high affinity (S1)
YDR075w	2	P12.1.f12.1	*PPH3*	protein Ser/Thr phosphatase
YDR342c	2	P33.1.f24.1	*HXT7*	high affinity hexose transporter
YER111c	2	P3.116.f2.1	*SWI4*	transcription factor
YHR179w	3	P2.54.f2.1	*OYE2*	NADPH dehydrogenase (old yellow enzyme)
YML028w	2	P3.76.f3.1	*TSA1*	thiol specific antioxidant
YML111w	2	P4.5.wf4.1	–	strong similarity to ubiquitination protein Bul1p
YMR115w	2	P2.201.f2.1	–	similarity to YKL133c
YNL134c	2	P3.59.f3.1	–	similarity to *C. carbonum toxD* gene
YNR019w	2	P2.151.f2.1	*ARE2*	acyl-CoA sterol acyltransferase
YOR326w	2	P37.1.f5.1	*MYO2*	myosin heavy chain
YPL088w	5	P8.1.wlf6.1	–	similarity to aryl alcohol dehydrogenases
YPL134c	2	P33.2.f7.1	–	similarity to ADP/ATP carrier proteins

matched the same *S. cerevisiae* ORF over different segments, we considered that we were dealing with a single orthologue for the minimal multiple matches set (minMMS), and with two orthologues (or more) for the maximum multiple matches set (maxMMS).

Applying the above rules, the minimal number of new genes in *S. servazzii* was estimated at 1410 (minMMS) and the maximal at 1586 (maxMMS) with high scores of matches (average 58% identity and 72% similarity). Assuming that the genomes of *S. cerevisiae* and *S. servazzii* are similar in gene content, we have identified about 25% of the nuclear genes of *S. servazzii*, not including tRNA genes. We found 46 nuclear tRNA genes in our search, equivalent to a 25% gene complement. Sequence conservation is high since E values ranged from 8×10^{-13} to 2×10^{-40}. The presence of introns within tRNA was also conserved even if variability in length and sequence of these introns was observed.

3.6. Duplicated genes

The number of recurrent matches within the minMMS amounted to 29 genes, listed in Table 1. Among these, 12 were singletons in *S. cerevisiae*, the remaining ones could be

Table 2
Identified spliceosomal introns in *S. servazzii*

RST	*S. cerevisiae* homologue	5′ splice site	Branchpoint	3′ site	Intron size (bp)	
					Ss	Sc
AT0AA011D08D1	YCR097w-1	AAA GTATGT	CAT TACTAAC ATW	TAG TTA	151	54
AT0AA011D08D1	YCR097w-2	TGG GTATGT	AAG TACTAAC CCT	TAG TTT	61	52
AT0AA003G02D1	YDL083c	CAA GTATGT	TAA TACTAAC AAG	CAG ACT	140	332
AT0AA008D03T1	YHR123w	CAG GTATGT	TTT TACTAAC CAG	CAG ATA	70	91
AT0AA001D01D1	YNL302c	TTA GTATGT	TTA TACTAAC AAA	CAG AGA	240	551
AT0AA004F02T1	YOR293w	AAG GTATGT	ATT TACTAAC ATW	TAG AAG	401	437
AT0AA016F09T1	YJL136c	TTA GTATGT	ATA TACTAAC MAT	TWG GTC	403	460
AT0AA012F01T1	YLR185w	TGG GTATGT	TTT TACTAAC AAC	TAG AGG	410	359
AT0AA013B05D1	YLR275w	GTC GTATGT	CTT TACTAAC AAT	CAG TGA	89	90
AT0AA012F04T1	YMR142c	TGG GTATGT	CAA TACTAAC ATA	TAG CTA	186	402
AT0AA005A04T1	YMR116c	AAR GTAGTT	TTT TACTAAC CAA	TAG GCT	356	273
AT0AA008G12D1	YPL129w	GCT GTATGT	GAA TACTAAC AAA	CAG ACT	176	106
AT0AA013H06D1	YIL069c	ATG GTATGT	GTA TACTAAC AAT	TAG TCT	520	410
AT0AA004G01D1	YGL178w	ATG GTATGT	TTT TWCTWWY WAY	CAG TTG	389	640
XAT0AA002D06D1	YML026c	na	TTT TACTAAC AAT	CAG TTT	na	463
XAT0AA002E03T1	YMR194w	na	TTT TACTAAC AAA	CAG GTA	na	401
AT0AA007D11T1	YLR426w	na	na	CAG TGG	na	71

The 5′ splice site column contains the last three nucleotides of the upstream exon and the canonical GTATGT 5′ splice site. The branchpoint column contains the last three nucleotides of the 5′ part of the intron, the canonical TACTAAC branchpoint and the first three nucleotides of the 3′ part of the intron. The 3′ site column contains the nucleotide preceding the canonical AG and the first three nucleotides of the downstream exon.

attributed to the gene families described in [20]. Genes present in paralogous gene families represent only 4.5% of the newly identified genes in *S. servazzii*. This is much less than what was observed in other species [21,22]. Among the *S. servazzii* paralogues identified here, ORFs involved in transport are the most frequent. Among the 12 genes that are duplicated in *S. servazzii*, and that are singletons in *S. cerevisiae*, two encode hypothetical proteins, three exhibit weak similarity to known proteins (two of these are regulatory proteins). The seven other genes belong to different functional categories: DNA repair, metabolism, mRNA transport, cytoskeleton organization (three homologues of *PAC2*), and initiation of DNA replication.

3.7. Orthologues with no equivalent in S. cerevisiae

We detected two significant matches (including that displayed by contig 409) to the RF4 protein from the *K. lactis* plasmid discussed above. We detected a match with a NAD(P)H dehydrogenase [quinone] 1 from human (SwissProt accession number P15559), which is involved in detoxification as well as in biosynthetic pathways. We found a significant match with a *Schizosaccharomyces pombe* protein, Mlo2, whose precise function is unknown but which interferes with mitotic chromosome segregation when overproduced. Both ORFs were also detected in some of the species studied in this project and were probably lost in *S. cerevisiae* [23]. Finally, a homologue to a second *S. pombe* ORF, SPAC8F11.02C, was detected. The limited number of novel genes detected by this approach is an indication of the close relationship between *S. servazzii* and *S. cerevisiae*. In addition, this implies that no recent horizontal transfer of genetic material has occurred.

3.8. Functional classification

We examined the functions of the *S. servazzii* genes orthologous to *S. cerevisiae* according to the MIPS functional catalogue modified [23]. Overall, the distribution of functions as defined in *S. cerevisiae* is not different when the two yeasts are compared. A clear deficit appears among genes involved in peroxisomal organization (class 30.19) and peroxisomal transport (class 8.10), amounting to half or a third, respectively, with reference to *S. cerevisiae*. Differences are also detectable in metabolic genes as several categories are significantly increased: over 150% for amino acid metabolism (classes 01.01.04 and 01.01.99), 179–194% for deoxyribonucleotide metabolism (classes 01.03.07 and 01.03.13), and 179% for lipid metabolism (class 01.06.99). A general increase was observed for an ensemble of functions involved in cellular communication and signal transduction (class 10) with respect to osmosensing, signal transduction activities, pheromone response and signal transduction (146–160% increase). Functional analysis of the subset of genes we have detected would indicate that the 'functional composition' does not deviate fundamentally from that of *S. cerevisiae* except that clear differences can be seen for some metabolic pathways and signal transduction activities (see [23]). This may reflect an adaptation of *S. servazzii* to a different ecological niche and could be linked to the limited ability of fermentation and assimilation of carbohydrate displayed by this organism.

3.9. Nuclear spliceosomal introns

Around 4% of *S. cerevisiae* genes carry spliceosomal introns. These are mostly located at the 5′ ends of genes, are short (< 400 bp) and unique [24]. Although the sequences available in our study are relatively short single reads, we attempted to define introns within *S. servazzii* genes homologous to intron carrying genes in *S. cerevisiae* using the MIPS database. We found 12 RSTs that contained the *S. cerevisiae* consensus sequences for the 5′ splice site, the branchpoint and the 3′ splice site. In three RSTs, the 5′ site was not present, and in one of these RSTs, only the 3′ site could be detected (Table 2). This was expected as homologous regions in intron containing genes will become visible at the 3′ site.

Consensus sequences and intron positions were identical to those of *S. cerevisiae* except for RST AT0AA001D01D1 with one base missing in the codon interrupted by the intron and for RST AT0AA016F09T1, which carries the canonical 5′ splice site sequence GTATGT instead of GTACGT found in *S. cerevisiae*. For the 3′ splice site, we found seven TAG and eight CAG sequences. Amongst the genes in which an intron was detected, seven out of 15 encode ribosomal proteins (88/324 in *S. cerevisiae*). Novel splice sites might have escaped detection, since no systematic search for introns was launched. Overall, one can conclude that splice sites and

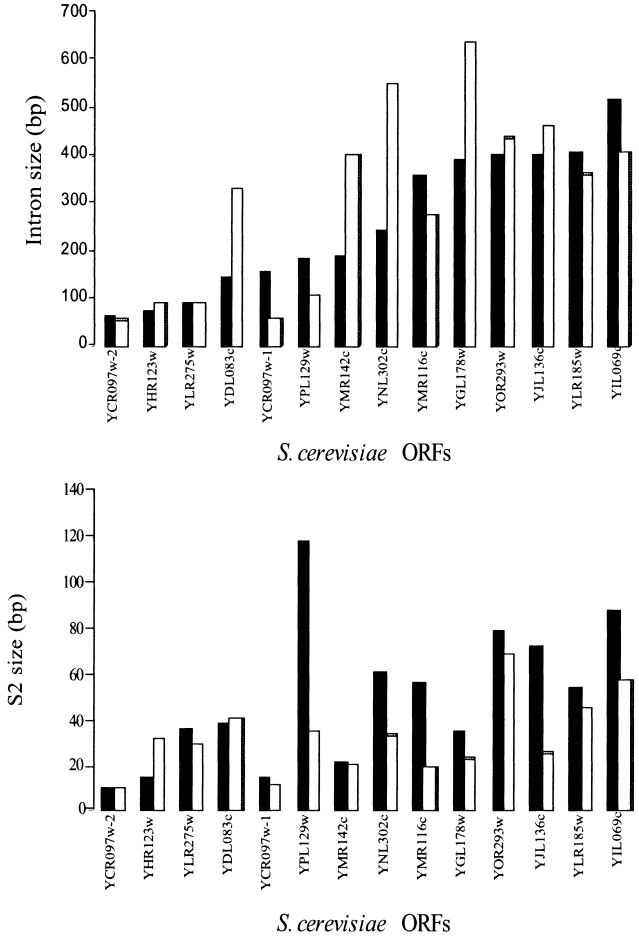

Fig. 1. Comparison of spliceosomal intron sizes between *S. cerevisiae* and *S. servazzii*. Intron size and S2 size in bp are plotted in relation to the intron containing *S. cerevisiae* ORFs. Open bar: *S. cerevisiae*, closed bar: *S. servazzii*. Top: whole intron size; bottom: S2 size (interval from the end of the branchpoint TACTAAC to the last position of the intron).

branchpoint sequences are remarkably conserved between *S. servazzii* and *S. cerevisiae*, indicating that the splicing mechanism is likely to be conserved between the two yeasts. For five genes homologous to *S. cerevisiae* (YDR129c, YPL031c, YFL034c-b, YDR381w, YBR119w), no intron was detected either at the corresponding location or elsewhere in the *S. servazzii* homologues. For 17 RSTs, the homologous regions within the RSTs were remote from the location of introns and therefore the introns were not present in the RSTs considered (data not shown). In Fig. 1, a comparison of the size of the entire intron and the interval between the branchpoint and the 3′ splice site, S2, for pairs of homologues is shown. Overall, sizes of introns are equivalent in both species, except for four cases with clearly longer introns in *S. cerevisiae*.

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