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Genomic Exploration of the Hemiascomycetous Yeasts: 6. *Saccharomyces exiguus*

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Abstract Random sequence tags were obtained from a genomic DNA library of *Saccharomyces exiguus*. The mitochondrial genome appeared to be at least 25.7 kb in size, with a different organization compared to *Saccharomyces cerevisiae*. An unusual putative 953 bp long terminal repeated element associated to Ty3 was found. A set of 1451 genes was identified homologous to *S. cerevisiae* open reading frames. Only five genes were identified outside the *S. cerevisiae* taxon, confirming that *S. exiguus* is phylogenetically closely related to *S. cerevisiae*. Unexpectedly, numerous duplicated genes were found whereas they are unique in *S. cerevisiae*. The sequences are deposited at EMBL under the accession numbers: AL407377–AL409955.

Key words: Retrotransposon; Duplicated gene; Mitochondrial DNA

1. Introduction

The yeast *Saccharomyces exiguus* belongs to the heterogeneous *Saccharomyces sensu lato* group known to possess a high potential for both basic and applied research [1,2]. From Japan to The Netherlands, *S. exiguus* species are found in soil, sewage, strawberries, grape must, sauerkraut, fermenting cucumbers or soft drinks [3]. Poorly characterized, *S. exiguus* is also referred to as *Cryptococcus holmii* (Jörgensen) Skinner, *Torula galactosa* Harrison, *Torula holmii* Jörgensen, and *Torulopsis holmii* (Jörgensen) Lodder var. *holmii*. The anamorph form (asexual state) is *Candida holmii* (Jörgensen) Meyer and Yarrow [1].

S. exiguus is a heterogeneous species [3]. Studies of 16 strains of *S. exiguus* have revealed a karyotype pattern different not only from that of *S. kluyveri* and *Saccharomyces cerevisiae*, but also from that of the other species included in the *Saccharomyces sensu lato* group [4,5]. In fact, there is no consensus on the exact chromosome number of this species. Depending on strains, chromosomes can be separated into 8–16 bands ranging in size from 290 to 2200 kb, leading to the distinction of four sub-groups of *S. exiguus* species [3]. The CBS379 type strain used in the present study exhibits 14–16 chromosomal bands [3], resulting in the largest genome size among *Saccharomyces* yeasts, i.e. 18 Mb [6,7]. However, since this *S. exiguus* strain is probably diploid, some bands may reflect heterozygosity [2]. This is also the only species among the *Saccharomyces sensu lato* that contains small chromosomes < 500 kb [6]. The other *S. exiguus* sub-groups are karyotypically and genetically different [2,3].

Available sequence data are scarce in *Saccharomyces sensu lato* species, notably for *S. exiguus*. To date, only 37 DNA sequences and 19 proteins have been released in GenBank. Most of these sequences concern nuclear ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA). The rest concern two autonomously replicating sequences, and only four nuclear genes encoding the orotidine 5′-phosphate decarboxylase [8], E-Ste3p, a homologue of the *S. cerevisiae* α-receptor gene [9], the homologue of Abp1p, an actin-binding protein [10], and the mating factor α [11]. In contrast to yeasts of the *sensu stricto* group, *S. exiguus* is a petite positive moderately inducible by ethidium bromide. Classical genetics is possible in *S. exiguus* although not developed. A transformation system has been developed, allowing gene disruption and extra-chromosomal gene amplification. Conditions for making compatible auxotrophs and tetrad dissection were described by Naumov et al. [2].

This article presents the comparative genomic analysis of the *S. exiguus* CBS379 type strain through the exploitation of 2579 random sequence tags (RSTs). Their annotation led to the identification of a minimal set of 1451 genes with homologues in *S. cerevisiae* plus five other genes absent from *S. cerevisiae*. Differences in gene order and function were also evaluated with respect to *S. cerevisiae*.

2. Materials and methods

2.1. Library construction

The diploid *S. exiguus* type strain CBS379 (CLIB179) was used in this study. DNA fragmentation and cloning, as well as the quality control of the library, were performed as described by Neuvéglise et al. [12]. A set of 1389 clones was sequenced by Génoscope [13]. The traces obtained were then treated and assembled as Tekaia et al. [14] reported.

2.2. Characteristics of the genomic DNA sequences

A total of 2579 RSTs of 923 ± 181 bp in size were obtained [13]. Around 86% of the RSTs had both ends sequenced, whereas the remaining had only one end sequenced. Assembly of the RSTs resulted in 228 contigs ranging from 0.83 kb to 7.2 kb in size. Most of them (91%) were composed of two or three sequences. The nuclear DNA (G+C) content was estimated at 32.92% which is in agreement

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with the value reported by Barnett et al. [1]. The average size of the inserts was found to be 3.59 ± 0.58 kb by enzymatic restriction analysis.

3. Results and discussion

3.1. rDNA

A set of two contigs was identified as rDNA. Contig 494 (7195 bp) was found to contain the entire 18S gene, ITS1, the 5.8S, ITS2, and the 26S gene, and at both extremities, the NTS1 and NTS2, were truncated and poorly conserved between the two species. The presence of the 5S gene plus part of the NTS2 was detected in contig 320 (1883 bp). In fine, more than 8 kb of rDNA was identified but it was not possible to deduce the size of the entire *S. exiguus* rDNA unit. Only 21 ribosomal units were estimated to occur in the *S. exiguus* genome which is low compared to *S. cerevisiae* in which 140 units are present [7]. A similar tendency has been reported in other yeast species studied in this project [12, 15–17]. These results could either be due to a bias in the library due to the use of *Cvi*JI enzyme, or to a different rate of metabolism with respect to *S. cerevisiae* as proposed for *Saccharomyces uvarum* [15]. One insert (AV0AA006E06) contained a match both to rDNA (18S) and to the homologue of YOR326w (MYO4). In *S. cerevisiae*, the gene organization at the junction is different since the open reading frame (ORF) upstream from the ribosomal cluster is YLR155c.

3.2. mtDNA

A set of 10 contigs (i.e. 208 RSTs) plus four isolated RSTs were identified (Table 1). Except *COX1* and *COB*, the other mitochondrial gene sequences appeared highly conserved in *S. exiguus*. As their counterparts in *S. cerevisiae*, the *S. exiguus COX1* and *COB* genes exhibit a mosaic intronic structure, but the ai1, ai2, ai5α, ai5β, and ai5γ introns of *COX1* [18] were not detected in *S. exiguus*. Similar observations have been reported for *S. kluyveri* and *S. servazzii* [12,16], suggesting a different organization of introns. Several intronic ORFs were also detected to be associated to *COX1, COB* and the 21S rRNA gene. No significant matches were found with the ORI/REP sequences of *S. cerevisiae*, suggesting that the origins of replication are not conserved in *S. exiguus*. In contrast, mitochondrial tRNA genes sequence ($n = 19$) appear conserved in *S. exiguus* with more than 93% of identity. Only five tRNA genes were not identified: tRNA_Val, Phe, Thr1,

Table 1
S. exiguus mtDNA organization compared to that of *S. cerevisiae*

Contig or RST	Position	Gene	Identity (%)	*S. cerevisiae* location
C487 (1534 bp)	1347–1419	tRNA_Thr2	97	63 935–63 849
C487	1245–1329	tRNA_Tyr	94	70 911–70 823
C487	1156–1216	tRNA_Asn	88	71 497–71 437
C487	1042–1116	tRNA_Ala	85	69 922–69 846
C487	787–858	tRNA_Ile	95	70 235–70 164
C487	590–674	tRNA_Ser	95	48 247–48 204
C487	154–531	15S	95	6958–6565
C481 (1191 bp)	102–629	15S	91	6546–8194
C488 (2163 bp)	1327–1398	tRNA_Pro	97	802–726
C488	1534–1601	tRNA_Pro anti	94	731–798
C489 (2321 bp)	450–1214	*ATP6*	83	28 487–29 266
C489	1660–1764	*ATP9*	83	46 722–46 943
C484 (2297 bp)	1649–918	*COX2*	90	73 803–74 413
C484	847–810	tRNA_Trp	94	9400–9437
C484	664–17	21S	84	58 022–58 704
C495 (2592 bp)	2237–709	21S	92	58 876–60 765
C495	442–25	I-*Sce*1	80	60 765–61 404
AV0AA009H11D1 (308 bp)	1–130	21S	86	61 977–62 106
C493 (3158 bp)	19–68	tRNA_Val1	89	64 438–64 488
C493	333–398	tRNA_Cys	92	78 531–78 601
AV0AA013B11D1	734–1495	*COX3*	85	79 214–79 947
C493	1585–2580	ORF1	28	74 495–75 984
C496 (4796 bp)	684–763	tRNA_Leu	95	66 092–66 175
C496	983–1048	tRNA_Gln	100	66 213–66 278
C496	1107–1178	tRNA_Lys	95	67 061–67 132
C496	1202–1274	tRNA_Arg1	95	67 306–67 383
C496	1282–1348	tRNA_Gly	94	67 464–67 535
C496	1454–1525	tRNA_Asp	90	68 322–68 393
C496	1531–1613	tRNA_Ser2	87	69 198–69 285
C496	1699–1765	tRNA_His	83	64 647–64 668
AV0AA013B11T1	1839–1903	tRNA_Glu	95	35 376–35 440
AV0AA013B11T1	2319–2774	*COB*	67	36 544–36 901
AV0AA013B11T1	2748–3552	*Scb*I2	68	37 939–38 597
C496	3962–4669	*COB*	77	40 866–43 586
C486 (2458 bp)	2018–1777	*COX1*	86	13 820–16 470
C486	1778–1325	putative ai3	–	–
C486	1326–908	*COX1*	88	20 514–20 932
C492 (3267 bp)	2239–1325	I-*Sce*II, ai4	83	21 186–21 903
C492	1221–346	*COX1*	86	21 990–26 697

Mitochondrial coding regions and RNA genes were identified using BlastX and BlastN comparisons against *S. cerevisiae* mitochondrial genome and its products. The vertical dashed lines indicate a physical link between two different contigs or between a contig and a single RST. The location of the matches in *S. cerevisiae* is also indicated [18].

Table 2
Occurrence of gene duplications in *S. exiguus* classified according to *S. cerevisiae* homologues

	Match	Gene number	*S. cerevisiae* unique/family		Match	Gene number	*S. cerevisiae* unique/family
A	YAL046c	2	S	I	YIL090w	2	S
					YIL106w	2	P2.343.f2.1
B	YBL004w	2	S				
	YBR008c	3	P12.2.f8.1	J	YJL171c	2	P2.332.f2.1
	YBR153w	2	S		YJL200c	2	P4.9.f4.1
	YBR203w	2	S		YJR033c	2	S
	YBR207w	2	P2.415.f2.1		YJR050w	2	S
	YBR245c	2	P17.1.f16.1				
				K	YKL004w	2	P2.315.f2.1
C	YCL017c	2	S		YKL089w	2	S
	YCR009c	2	P4.29.f2.1		YKR048c	2	S
					YKR051w	2	S
D	**YDL080c**	2	P7.9.f5.1				
	YDL081c	2	P2.449.f2.1	L	YLR034c	2	P3.43.f3.1
	YDR069c	2	P10.4.f6.1		YLR087c	2	S
	YDR071c	2	S		YLR409c	2	S
	YDR389w	2	P2.108.f2.2				
	YDR425w	2	P2.433.f2.1	M	YMR021c	2	S
					YMR031c	2	P2.209.f2.1
E	YEL004w	2	S				
	YEL021w	2	S	N	YNL051w	2	S
	YEL046c	2	S		YNL081c	2	S
	YER005w	2	P2.423.f2.1		YNL212w	2	S
	YER015w	2	P4.38.f4.1		YNR043w	2	S
	YER016w	2	S				
	YER065c	2	P2.31.f2.1	O	YOL087c	2	S
	YER171w	2	P2.76.f2.1		YOL098c	2	P2.135.f2.1
					YOR042w	2	S
F	**YFL034cb**	2	P2.343.f2.1		YOR126c	2	S
	YFL034w	2	S		**YOR243c**	2	S
	YFR031c	2	P37.1.f4.1		**YOR244w**	2	P3.31.f3.1
					YOR245c	2	S
G	YGL207w	2	S		YOR254c	2	S
	YGL216w	2	P37.1.f5.2				
	YGL224c	2	P2.398.f2.1	P	YPL262w	2	S
	YGR080w	2	S		YPR021c	2	P33.2.f7.1
	YGR081c	2	S		YPR122w	3	P2.13.f2.1
					YPR141c	2	P37.1.f5.2
H	YHL007c	2	P108.1.f3.3		YPR172w	2	P2.3.f2.1
	YHR120w	2	P6.6.f5.1				
	YHR124w	2	S				
	YHR205w	2	P108.1.f16.1				

Only non-ambiguous matches were considered (matches 'o', see [14]). NB: Bold fonts underline the existence of several series of syntenic ORFs found in multiple copies in *S. exiguus*. S: Genes occurring in a single copy in *S. cerevisiae*; P: genes in a *S. cerevisiae* family.

Met1 and Met2. Two copies of the tRNA_Pro were identified in contig 488, one in the orientation found in *S. cerevisiae*, and the other one in the opposite orientation, which latter was termed tRNA_Pro anti. Concerning mitochondrial ribosomal RNAs, we identified the 15S gene and the 21S gene with part of its omega intron.

Taken together, the 10 contigs represent around 25.7 kb which is more than the 23 kb previously estimated by Piskur et al. [19] for the *S. exiguus* mitochondrial genome. The mtDNA size of *S. exiguus* probably exceeds 25.7 kb since some coding regions and tRNA genes were not identified in the present study. This size could be very similar to that observed in *S. servazzii* and *S. unisporus* (29 kb), or *S. castellii* (26 kb) [20]. However, *S. exiguus* shows a mitochondrial gene organization (Table 1) deviant from these species and *S. cerevisiae* [18], revealing the existence of a variability in the physical organization of the mtDNA among the *Saccharomyces* species. The (G+C) content was estimated to be around 26.3% but could be lower since some (A+T)-rich intergenic regions were missing.

3.3. Transposable elements

Retrotransposons in *S. exiguus* have been searched for by BlastX comparisons to the coding sequence of the five classes of retroelements in *S. cerevisiae*. As the Ty5 element is truncated in *S. cerevisiae*, the entire Ty5 of *S. paradoxus* was used to screen our DNA library.

A set of 47 RSTs were found to contain part of Ty proteins. Ty1- and Ty2-like elements were identified in contig 490 (52% identity at the amino acid level compared to *S. cerevisiae*). Delta (δ) long terminal repeat (LTR)-like sequences of 424 bp, including 5 bp perfect inverted repeats (5′-TGACG.../...CGTCA-3′), were identified. When these latter were located at the 5′ end of a Ty1-like sequence, an ORF of 432 bp encoding a putative protein of 144 codons was detected, starting 44 bp upstream from the 3′ extremity of the δ LTR-like sequence. This ORF may correspond to a *TYA* sequence which in *S. cerevisiae* begins 39 bp inside the upstream δ sequence. When associated with the 3′ end of the sequence of TY1B, it appeared 32 bp downstream from this. Two solo δ LTR-like sequences flanked by the 5 bp repeat 'ACCAT' or

'ATAAT' were found in two isolated RSTs, representing the target site duplication.

No match to the Ty4 element was found in our genome survey. However, 10 RSTs contained an ORF homologue to the *S. paradoxus* Ty5 protein (48% identity). Ty5 LTR-like sequences have also been observed in six RSTs, consisting of a 370 bp sequence which includes a 5 bp perfect inverted repeat (5′-TGTTG.../...CAACA-3′). In *S. paradoxus*, Ty5 LTRs are 250 bp in length [21]. When this repeated sequence was present as a solo element (two cases), it was flanked by 5 bp direct repeats, ATCAG or ATCTT, respectively, indicative of the target site duplication.

A total of 19 RSTs containing a homologue of the *S. cerevisiae* TY3B protein were identified in *S. exiguus*. TY3B-like proteins appear less conserved in *S. exiguus* than TY1B- or TY2B-like proteins, notably in their N- and C-termini. In higher eukaryotes, *gypsy*-associated LTRs vary considerably in length from several hundred base pairs to more than 2 kb [22]. Whereas only short LTRs (from 332 to 427 bp) were initially detected in fungi, long LTRs of the *gypsy* type retrotransposon have been reported in *Yarrowia lipolytica* (714 bp) [23]. In *S. exiguus*, we found two RSTs that display a highly conserved sequence of 953 bp (contig 491) and 27 RSTs containing part of this sequence. This repeated element was found associated to TY3B or as a solo element. In both cases the conserved DNA sequence contains a 6 bp perfect inverted repeat (5′-TGTAAC.../...GTTACA-3′). If present as a solo element, it is flanked by a 5 bp duplication of the genomic target site 'TAATA'. In six cases, the TY3-like element was found associated to a tRNA gene, located between 11 and 19 bp upstream. The distribution of Ty elements in *S. cerevisiae* genome is clearly non-random: most Ty3 insertions are found closely associated to tRNA genes being located 16–19 bp upstream [24]. Thus, the association of the Ty3-like element with tRNA genes as observed here could again reflect the general influence of tRNAs genes on transposition and the intricate ways in which these transposable elements and their host must have co-evolved [24].

3.4. Identification of homologues

Repeated sequences such as rDNA, mtDNA, and Ty elements were removed from the total set of the RSTs, prior to the subsequent comparisons. Nearly 2/3 of the RSTs (1553) revealed significant matches with sequences in the *S. cerevisiae* proteome database. The average length of the alignments is 154 amino acids, with a high percentage of amino acid identity/similarity (i.e. 56%/71%). On the other hand, 1/3 of the RSTs did not have any significant match with the sequences in the *S. cerevisiae* database. Interestingly, the entire sequence of 52 ORFs ranging from 57 to 290 codons became available. Taking into account the fact that different RSTs may match different or overlapping parts of the same target [14], we finally estimated the minimal number of genes identified in *S. exiguus* to amount to 1451 and the maximal number to 1590.

Table 3
Functional distribution of ORFs found in one copy in *S. cerevisiae* and existing in multiple copies in *S. exiguus*

ORF	Gene	Copies	MIPS functional classification
YBR153w	RIB7	2	Oxidoreductase, HTP reductase
YCL017c	NFS1	2	Complex assembly protein, involved in tRNA-processing and mitochondrial metabolism
YEL004w	YEA4	2	Strong similarity to *Kluyveromyces lactis* Golgi uridine diphosphate-*N*-acetylglucosamine transporter
YEL021w	URA3	2	Orotidine-5′-phosphate decarboxylase
YEL046c	GLY1	2	L-Threonine aldolase, low-specific
YER016w	BIM1	2	Binding to microtubules
YGL207w	SPT16	2	General chromatin factor
YGR080w	TWF1	2	Protein kinase, transferase, an actin monomer sequestering protein
YHR124w	NDT80	2	Transcription factor, meiosis-specific protein
YKL089w	MIF2	2	Required for normal chromosome segregation and spindle integrity
YKR048c	NAP1	2	Complex assembly protein nucleosome assembly protein I
YMR021c	MAC1	2	Metal-binding activator
YNL081c		2	RNA-binding protein, similarity to ribosomal protein S13
YNR043w	MVD1	2	Lyase, mevalonate pyrophosphate decarboxylase
YOR126c	IAH1	2	Hydrolase, isoamyl acetate hydrolytic enzyme
YOR254c	SEC63	2	Chaperones, ER protein-translocation complex subunit
YPL262w	FUM1	2	Fumarate hydratase
YAL046c		2	Protein of unknown function
YBL004w		2	Weak similarity to *Papaya ringspot* virus polyprotein
YBR203w		2	Hypothetical protein
YDR071c		2	Similarity to *O. aries* arylalkylamine *N*-acetyltransferase
YFL034w		2	Similarity to hypothetical *S. pombe* protein and to *C. elegans* F35D11 protein
YGR081c		2	Weak similarity to mammalian myosin heavy chain
YIL090w		2	Similarity to hypothetical *S. pombe* protein
YJR033c		2	Similarity to *Drosophila* DmX gene
YJR050w	UTR3	2	Protein of unknown function
YKR051w		2	Similarity to *C. elegans* hypothetical protein
YLR087c		2	Hypothetical protein
YLR409c		2	Strong similarity to *S. pombe* β-transducin
YNL051w		2	Hypothetical protein
YNL212w		2	Weak similarity to *C. cardunculus* cypro4 protein
YOL087c		2	Similarity to *S. pombe* hypothetical protein
YOR042w		2	Weak similarity to YDR273w
YOR243c		2	Similarity to *Methanococcus jannaschii* hypothetical protein MJ0588
YOR245c		2	Similarity to hypothetical *C. elegans* proteins

All RSTs were finally compared to a protein database containing non-*S. cerevisiae* sequences exclusively [14]. Only five significant hits were obtained, but with low alignment scores. Two *Caenorhabditis elegans* homologues, one encoding a hypothetical protein (CEZK753.3) and the other one a protein similar to a sphingomyelin phosphodiesterase (CEZK455.4), were identified, as well as two *Schizosaccharomyces pombe* homologues corresponding to hypothetical proteins (AC26A3.14C, AC8F11.02C). Additionally, we found a homologue of the rat liver NAD(P)H dehydrogenase (quinone) (P05982). This flavoprotein enzyme apparently serves as a quinone reductase in detoxification pathways as well as in biosynthetic processes in the liver [25]. In total, we identified a minimum of 1456 genes in *S. exiguus*.

3.5. Comparison of gene redundancy in S. exiguus and S. cerevisiae

Based on the minimal set of *S. cerevisiae* homologues, it appears that 13% of the 1451 genes found in *S. exiguus* are present in multiple copies. 79 ORFs are duplicated and three ORFs are triplicated. Among these 82 *S. exiguus* redundant genes, 35 exist in a single copy in *S. cerevisiae*, the others belong to gene families. With reference to *S. cerevisiae* chromosomes, the duplicated genes in *S. exiguus* mainly corresponded to those found in chromosomes V, XIV and XV (Table 2). Sometimes, synteny appeared to be conserved, such as in chromosome XV, in which a series of three syntenic ORFs (homologous to YOR243c, YOR244w and YOR245c, respectively) are duplicated in *S. exiguus* (Table 2). Two of these ORFs are singletons in *S. cerevisiae*.

3.6. Functional classification of orthologues

A minimum of 1456 *S. exiguus* genes were identified in this work. The functions of the corresponding ORFs were evaluated through the assignment of their homologues in *S. cerevisiae*, based on the Functional Catalogue of MIPS as modified by Gaillardin et al.[26], and assuming that the *S. exiguus* genes have conserved the same functions as their *S. cerevisiae* counterparts.

The results show that the functional distribution of the newly identified genes is globally conserved between both these species. However, some functional sub-categories seem slightly over-represented probably due to different levels of gene duplications in *S. exiguus* versus *S. cerevisiae*. Indeed, 43% of the redundant genes identified in *S. exiguus* exist in a single copy in *S. cerevisiae* (Table 3). Half of these duplicated genes appear preferentially involved in cellular organization and cell growth (cycle control, mitosis, meiosis, budding, cell polarity, and filament formation), in metabolism (metabolism of vitamins, lipids, nucleotide as well as carbohydrate transport and utilization), and to a lesser extent in transcription, cellular biogenesis, protein destination, transport facilitation and other sub-categories. The remaining ones have no known function.

Interestingly, three of the duplicated genes displayed strong homologies to human disease-associated genes. This concerned the orthologues of the BIM1, MDR1 and FUM1 genes. Their products are the yeast homologues of human EB1 protein, oncoprotein Tre2 and Fum1 protein. These ubiquitous proteins are involved in familial sporadic forms of colorectal cancer [27], rare childhood diseases affecting bone and muscles known [28], and in fumaric aciduria and encephalopathy [29], respectively.

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