J. Gen. Appl. Microbiol., **60**, 7−12 (2014)
doi 10.2323/jgam.60.7

jGAM

Full Paper

The early diverging ascomycetous budding yeast *Saitoella complicata* has three histone deacetylases belonging to the Clr6, Hos2, and Rpd3 lineages

(Received July 17, 2013; Accepted September 22, 2013)

Hiromi Nishida,[1,*] Takashi Matsumoto,[2] Shinji Kondo,[3] Makiko Hamamoto,[4] and Hirofumi Yoshikawa[2,5]

[1] *Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, Imizu, Toyama 939−0398, Japan*
[2] *Genome Research Center, Nodai Research Institute, Tokyo University of Agriculture, Tokyo 156−8502, Japan*
[3] *National Institute of Polar Research, Tokyo 190−8518, Japan*
[4] *Department of Life Sciences, School of Agriculture, Meiji University, Kanagawa 214−8571, Japan*
[5] *Department of Bioscience, Tokyo University of Agriculture, Tokyo 156−8502, Japan*

We sequenced the genomic DNA and the transcribed RNA of the ascomycetous budding yeast *Saitoella complicata*, which belongs to the earliest lineage (Taphrinomycotina) of ascomycetes. We found 3 protein-coding regions similar to Clr6 of *Schizosaccharomyces* (a member of Taphrinomycotina). Clr6 has a structure similar to that of Rpd3 and Hos2 of *Saccharomyces*. These proteins belong to the class 1 histone deacetylase (HDAC) family. The phylogenetic tree showed that the Clr6, Hos2, and Rpd3 lineages are separated in fungal HDACs. Basidiomycetes have 3 proteins belonging to the Clr6, Hos2, and Rpd3 lineages. On the other hand, whereas ascomycetes except for *Schizosaccharomyces* have the Hos2 and Rpd3 homologs, and lack the Clr6 homolog, *Schizosaccharomyces* has the Clr6 and Hos2 homologs, and lacks the Rpd3 homolog. Interestingly, *Pneumocystis* and *Saitoella* have 3 proteins belonging to the Clr6, Hos2, and Rpd3 lineages. Thus, these fungi are the first ascomycete found to possess all 3 types. Our findings indicated that Taphrinomycotina has conserved the Clr6 protein, suggesting that the ancestor of Dikarya (ascomycetes and basidiomycetes) had 3 proteins belonging to the Clr6, Hos2, and Rpd3 lineages. During ascomycete evolution, Pezizomycotina and Saccharomycotina appear to have lost their Clr6 homologs and *Schizosaccharomyces* to have lost its Rpd3 homolog.

Key words: ascomycetes; basidiomycetes; histone deacetylase; *Saitoella complicata*; *Schizosaccharomyces*

Introduction

The subphylum Taphrinomycotina (“Archiascomycetes”) is the earliest ascomycetous lineage that diverged before the separation of the subphyla Pezizomycotina (“Euascomycetes”) and Saccharomycotina (“Hemiascomycetes”) (Nishida and Sugiyama, 1993; An et al., 2002; Liu et al., 2009; Schoch et al., 2009). Genome analyses of ascomycetes belonging to Taphrinomycotina are central to the elucidation of ascomycetes evolution. At present, genome information on Taphrinomycotina is limited to that of species belonging to the genus *Schizosaccharomyces* (Wood et al., 2002; Rhind et al., 2011). The anamorphic and saprobic yeast *Saitoella complicata* is a member of Taphrinomycotina which has been isolated from Himalayan soil (Goto et al., 1987). Although *Schizosaccharomyces* is a fission yeast, *Saitoella* is a budding yeast. *Saitoella complicata* shares some characteristics with both ascomycetous and basidiomycetous yeasts (Sugiyama et al., 1985; Goto et al., 1987). For example, a negative diazonium blue B reaction and negative extracellular deoxyribonuclease activity are characteristics of ascomycetous yeasts, whereas positive urease activity, the major ubiquinone system Q-10, and enteroblastic budding are traits of basidiomycetous yeasts (Sugiyama et al., 1985; Goto et al., 1987). Based on 18S ribosomal DNA sequence comparison, *Saitoella complicata* forms a monophyletic lineage with *Schizosaccharomyces pombe* and *Taphrina*

*Corresponding author: Hiromi Nishida, Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939−0398, Japan
E-mail: hnishida@pu-toyama.ac.jp

None of the authors of this manuscript has any financial or personal relationship with other people or organizations that could inappropriately influence their work.

wiesneri (Nishida and Sugiyama, 1993). The genus *Saitoella* consisted solely of *Saitoella complicata* until *Saitoella coloradoensis* was reported in 2012 (Kurtzman and Robnett, 2012).

In a previous study, we showed an initial genome assembly of *Saitoella complicata* comprising 7,981 contigs (12,981,880 bp) (Nishida et al., 2011), in which most DNA sequences encode fragmented genes. A fragmented amino acid sequence similarity search showed that approximately 65% of the putative amino acid sequences had the highest similarity with proteins of Pezizomycotina (Nishida et al., 2011). Only 11.6% of the putative amino acid sequences had the highest similarity with proteins of *Schizosaccharomyces* (Nishida et al., 2011). To elucidate the characteristics of the *Saitoella* genomic DNA sequence, we refined the genome assembly with additional genomic DNA sequencing and annotated coding genes based on RNA sequencing using the Illumina HiSeq sequencing system.

In order to discuss phylogenetic relationships among Taphrinomycotina, we searched for homologs to Clr6 of *Schizosaccharomyces pombe* or Rpd3 of *Saccharomyces cerevisiae* and homologs to Elp3 in the *Saitoella compicata* genome, because the evolutionary conservation level of Clr6 (or Rpd3) is the highest among fungal histone deacetylases (HDACs) and Elp3 is the highest among histone acetyltransferases (HATs) (Nishida, 2009). Eukaryotic genomic DNA is packaged with histone proteins to form chromatin (Kornberg, 1977; Igo-Kemenes et al., 1982). The precise organization of the chromatin is important for the maintenance of the genomic DNA. Histones are generally post-translationally modified. Reversible histone acetylation, which is regulated by HAT and HDAC, is one such modification (de Ruijter et al., 2003; Lee and Workman, 2007; Yang and Seto, 2008). In addition to the maintenance of genomic DNA, the acetylation and deacetylation of histones play an important role in the regulation of transcription (Li et al., 2007; Luger and Richmond, 1998). HDACs are evolutionarily more ancient than their histone substrates and are phylogenetically divided into 3 main classes: Hos2- and Rpd3-like (class 1) proteins, Hda1-like (class 2) proteins, and Sir2-like (class 3) proteins (Ekwall, 2005). Class 1 HDACs are crucial for transcriptional repression and epigenetic landscaping (Yang and Seto, 2008). For example, Clr6 of *Schizosaccharomyces*, Hos1, Hos2, and Rpd3 of *Saccharomyces* belong to this class. However, the phylogenetic position of Hos1 is distant from those of Clr6, Hos2, and Rpd3 (Bjerling et al., 2002). In this study, we analyzed proteins similar to Clr6, Hos2, and Rpd3.

Materials and Methods

Saitoella complicata culture. *Saitoella complicata* NBRC 10748 (= JCM 7358, = IAM 12963; type strain) was used in this study. After the strain was cultivated in YM broth (3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g dextrose, and 1 L water) at 25°C for 3 days, the cells were washed 3 times with Tris-ethylenediaminetetraacetic acid buffer (pH 8).

DNA and RNA sequencing. Genome sequencing was performed and the complementary DNAs (cDNAs) from total messenger RNAs (mRNAs) were sequenced using the Illumina HiSeq sequencing system. The read pairs were dereplicated by FULCRUM (Burriesci et al., 2012) and assembled using the SOAPdenovo assembler (Li et al., 2010). The DNA Data Bank of Japan Supercomputer System was used for mapping of the Illumina reads to the genomes and some related computation.

High-throughput RNA sequencing-based gene prediction. Using AUGUSTUS (Stanke et al., 2008), a gene prediction software program based on the alignment of expressed sequences with the genome, we determined the coding exons (open reading frames) of the genes expressed on the assembled genome of *Saitoella* according to the gene model of *Schizosaccharomyces pombe* (a member of Taphrinomycotina), which is thought to have some taxonomic proximity to *Saitoella*. We obtained a total of 124,350,706 read pairs (100-bp × 2) by sequencing cDNAs from the total mRNAs of *Saitoella* with the Illumina HiSeq sequencing system. Of the read pairs, 114,441,522 were aligned uniquely to the genome using the BLAT (Kent, 2002). Based on the coordinates mapped by the expressed sequences, AUGUSTUS predicted 10,253 genes on the genome. We searched for amino acid sequences similar to Clr6 and Elp3 of *Schizosaccharomyces pombe* using BLAST (Altshul et al., 1990).

Phylogenetic analysis. BLAST (Altshul et al., 1990) was used to search for amino acid sequences similar to Clr6 of *Schizosaccharomyces pombe*. We selected the following 29 genomes of Dikarya (asomycetes and basidiomycetes): 7 genomes from Saccharomycotina (*Candida tropicalis*, *Debaryomyces hansenii*, *Kluyveromyces lactis*, *Lodderomyces elongisporus*, *Saccharomyces cerevisiae*, *Vanderwaltozyma polyspora*, and *Yarrowia lipolytica*), 9 genomes from Pezizomycotina (*Aspergillus nidulans*, *Botryotinia fuckeliana*, *Coccidioides immitis*, *Nectria haematococca*, *Neurospora crassa*, *Penicillium chrysogenum*, *Phaeosphaeria nodorum*, *Podospora anserina*, and *Tuber melanosporum*), 4 genomes from Taphrinomycotina (*Pneumocystis jirovecii*, *Saitoella complicata*, *Schizosaccharomyces pombe*, and *Taphrina deformans*), and 9 genomes from Basidiomycota (*Coprinopsis cinerea*, *Cryptococcus neoformans*, *Laccaria bicolor*, *Malassezia globosa*, *Moniliophthora perniciosa*, *Postia placenta*, *Puccinia graminis*, *Schizophyllum commune*, and *Ustilago maydis*). Multiple alignments were generated using MUSCLE (Edgar, 2004). Gap positions were not considered. A maximum likelihood tree was reconstructed using MEGA version 5 (Tamura et al., 2011). We used the general reverse transcriptase model (Dimmic et al., 2002) as the best amino acid substitution model based on the Bayesian information criterion score using MEGA version 5. The nearest neighbor interchange was used as the maximum likelihood heuristic method. The initial tree was automatically generated. The γ-distributed rate was considered and the number of discrete gamma categories was 5. The bootstrap was performed with 100 replicates.

In addition, BLAST was used to search for amino acid sequences similar to Elp3 of *Schizosaccharomyces pombe*. We used 69 fungal genomes. The phylogenetic analysis was performed as described above.

Genome-wide protein similarity search. The BLAST program was downloaded from the National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov). The protein sequence data of *Aspergillus oryzae* (Pezizomycotina, Ascomycota), *Mixia osmundae* (Pucciniomycotina,

Basidiomycota), *Pneumocystis jirovecii* (Taphrinomycotina, Ascomycota), *Saccharomyces cerevisiae* (Saccharomycotina, Ascomycota), *Schizosaccharomyces pombe* (Taphrinomycotina, Ascomycota), *Taphrina deformans* (Taphrinomycoita, Ascomycota), *Tuber melanosporum* (Pezizomycotina, Ascomycota), *Ustilago maydis* (Ustilaginomycotina, Basidiomycota), and *Yarrowia lipolytica* (Saccharomycotina, Ascomycota) were also downloaded from the NCBI BioProjects PRJNA28175, PRJDA48573, PRJEA68827, PRJNA128, PRJNA127, PRJEA74523, PRJNA49017, PRJNA14007, and PRJNA12414, respectively. Recently, the Taphrinomycotina draft genomes of *Pneunocystis jirovecii* (Cisséa et al., 2012) and *Taphrina deformans* (Cisséa et al., 2013) were reported. There is a hypothesis that the earliest diverging fungi in the Pezizomycotina and Saccharomycotina have the proteins similar to Taphrinomycotina proteins. These fungi were included in this study. The class Pezizomycetes is the earliest diverging lineage in the Pezizomycotina (Lutzoni et al., 2004; Reeb et al., 2004). *Tuber melanosporum* belongs to the Pezizomycetes. *Yarrowia lipolytica* is the earliest diverging lineage in the Saccharomycotina (Wang et al., 2009). The BLASTP was used to search proteins with *E*-value$<$0.001. Among the similar proteins for each query protein, the protein with the lowest score was collected.

Results

We generated a total of 11,384,538 read pairs (100-bp × 2) by sequencing the genomic DNA of *Saitoella complicata* using the Illumina HiSeq sequencing system. Assembly of the sequence data resulted in 1,800 contigs (14,220,909 bases). Those 1,800 DNA sequences were deposited in the DNA Data Bank of Japan, under the accession numbers BACD02000001−BACD02001800.

In order to elucidate the relationships between Taphrinomycotina and Pezizomycotina, we performed a genome-wide protein similarity search among 4 species of Taphrinomycotina (*Pneunocystis jirovecii, Saitoella complicata, Schizosaccharomyces pombe, and Taphrina deformans*), 2 species of Saccharomycotina (*Saccharomyces cerevisiae* and *Yarrowia lipolytica*), 2 species of Pezizomycotina (*Aspergillus oryzae* and *Tuber melanosporum*), and 2 species of Basidomycota (*Mixia osmundae* and *Ustilago maydis*). As the results, 3,357, 5,955, 4,786, and 4,198 proteins of *Pneumocystis*, *Saitoella*, *Schizosaccharomyces*, and *Taphrina* exhibited the highest similarity to the other 9 fungal proteins, respectively (Table 1). The 25.9%, 46.7%, 25.3%, and 28.9% of the *Pneumocystis*, *Saitoella*, *Schizosaccharomyces*, and *Taphrina* proteins showed the highest similarity to proteins of Pezizomycotina, respectively (Table 1). It showed that not only the *Saitoella complicata* proteins but also 3 other Taphrinomycotina proteins exhibited higher similarity to proteins of Pezizomycotina than those of Saccharomycotina (Table 1).

We found 1 protein-coding region (g2957) similar to Elp3 of *Schizosaccharomyces pombe* in the *Saitoella complicata* genome using BLAST. The phylogenetic tree showed that Dikarya (ascomycetes and basidiomycetes) have a single Elp3, except for *Puccinia graminis* (Fig. 1). The phylogenetic tree showed that Ascomycota and Basidiomycota are separated and Ascomycota consists of Pezizomycotina, Saccharomycotina, and Taphrinomycotina (Fig. 1). However, Taphrinomycotina was separated into 2 lineages, the *Saitoella-Taphrina* lineage and the *Pneumocystis-Schizosaccharomyces* lineage, with low bootstrap support (Fig. 1).

On the other hand, we found 3 protein-coding regions (g336, g6187, and g6226) similar to Clr6 of *Schizosaccharomyces pombe* in the *Saitoella complicata* genome using BLAST. Although we found 3 proteins similar to the *Schizosaccharomyces* Clr6 in *Pneumocystis jirovecii*, we did not find any similar protein in *Taphrina deformans*. The phylogenetic tree showed that Dikarya have the Clr6, Hos2, and Rpd3 lineages (Fig. 2). Although the Hos2 and Rpd3 lineages had high bootstrap support (99% and 95%, respectively), the Clr6 lineage had low support (27%). The phylogenetic position of *Saitoella complicata* g336 is located near those of *Pneumocystis jirovecii* gi430812636 and *Schizosaccharomyces pombe* Clr6 (Fig. 2). Those of g6187 and g6226 are located in the Hos2 and Rpd3 lineages, respectively (Fig. 2).

Basidiomycetes have the 3 types of Clr6, Hos2, and Rpd3 homologs. On the other hand, Pezizomycotina and Saccharomycotina of ascomycetes lack the Clr3-like protein and *Schizosaccharomyces* lacks the Rpd3-like protein. Thus, *Pneumocystis jirovecii* and *Saitoella complicata* are the first ascomycetes found to possess all 3 of the Clr6, Hos2, and Rpd3 homologs.

Discussion

The gene content of the *Saitoella complicata* genome has higher affinity to Pezizomycotina than to Saccharomycotina

Table 1. Number of fungi with which each protein of query species had the highest similarity using BLASTP.

Query	*Pneumocystis*	*Saitoella*	*Schizosaccharomyces*	*Taphrina*
Pneumocystis		590 (9.9%)	603 (12.6%)	393 (9.4%)
Saitoella	1,244 (37.1%)		1,413 (29.5%)	1,669 (39.8%)
Schizosaccharomyces	447 (13.3%)	475 (8.0%)		398 (9.5%)
Taphrina	558 (16.6%)	1,248 (21.0%)	863 (18.0%)	
Aspergillus	291 (8.7%)	1,179 (19.8%)	577 (12.1%)	645 (15.4%)
Tuber	577 (17.2%)	1,600 (26.9%)	627 (13.1%)	566 (13.5%)
Saccharomyces	41 (1.2%)	68 (1.1%)	174 (3.6%)	30 (0.7%)
Yarrowia	86 (2.6%)	321 (5.4%)	334 (7.0%)	200 (4.8%)
Mixia	52 (1.5%)	233 (3.9%)	95 (2.0%)	161 (3.8%)
Ustilago	61 (1.8%)	241 (4.0%)	100 (2.1%)	136 (3.2%)
Total	3,357	5,955	4,786	4,198

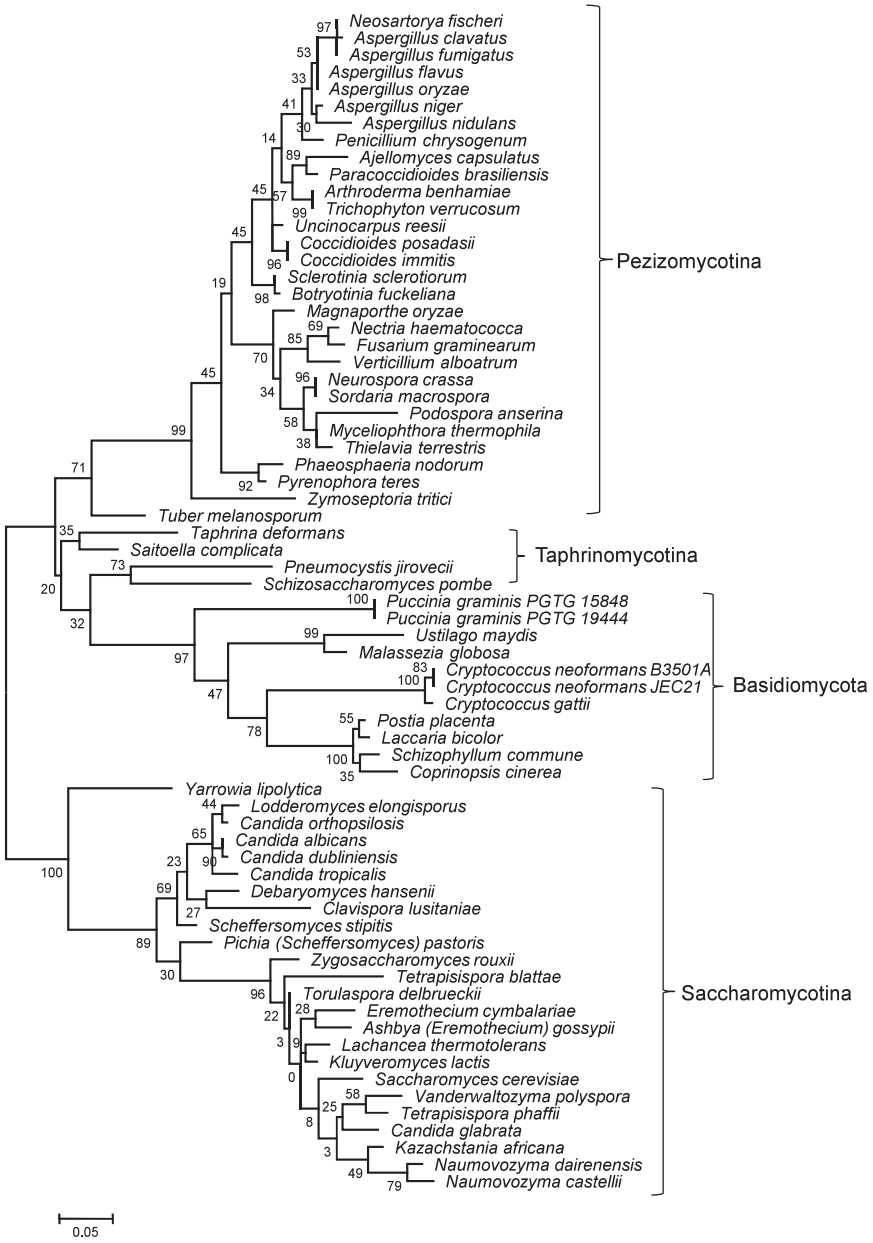

Fig. 1. Phylogenetic relationships among Elp3 and their homologous proteins.

Multiple alignments were generated using MUSCLE (Edgar, 2004). The maximum likelihood tree was reconstructed using MEGA version 5 (Tamura et al., 2011). The general reverse transcriptase model (Dimmic et al., 2002) was used as the amino acid substitution model. The nearest neighbor interchange was used as the maximum likelihood heuristic method. The γ-distributed rate was considered and the number of discrete gamma categories was 5. The bootstrap was performed with 100 replicates.

or *Schizosaccharomyces*. Based on a comparison of ribosomal RNA, RNA polymerase, and β-tubulin genes, the genus *Saitoella* is more closely related to the lineage of the 2 genera *Protomyces* and *Taphrina* than to that of other genera including *Schizosaccharomyces* (Sugiyama et al., 2006). These relationships were also found in a comparison of ribosomal RNA, RNA polymerase, and translation elongation factor genes (Kurtzman and Robnett, 2013). Ascospores of *Protomyces* and *Taphrina* can grow as budding yeasts in culture and have characteristics similar to those of *Saitoella* (Fonseca and Rodrigues, 2011; Kurtzman, 2011; Sugiyama and Hamamoto, 2011). To elucidate the phylogenetic relationships among Taphrinomycotina, genome sequences of Archaeorhizomycetes and Neolectomycetes are needed.

We showed that the Taphrinomycotina proteins exhibited higher similarity to proteins of Pezizomycotina than those of Saccharomycotina (Table 1). The results showed that the Taphrinomycotina genetic characteristics show greater similarity to those of filamentous ascomycetes than to those of ascomycetous yeasts. The results also support the notion that ancestral ascomycetes were filamentous and that hyphal growth was lost independently (Liu and Hall, 2004).

Taphrinomycotina is the earliest ascomycetous lineage that diverged before the separation of the subphyla Pezizomycotina and Saccharomycotina (Nishida and Sugiyama, 1993; An et al., 2002; Liu et al., 2009; Schoch et al., 2009). Thus, the genome information of Taphrinomycotina may retain the ancestral character of ascomycetes. In this study, we used HDACs similar to Clr6 of *Schizosaccharomyces* or Rpd3 of *Saccharomyces* as phylogenetic markers for a

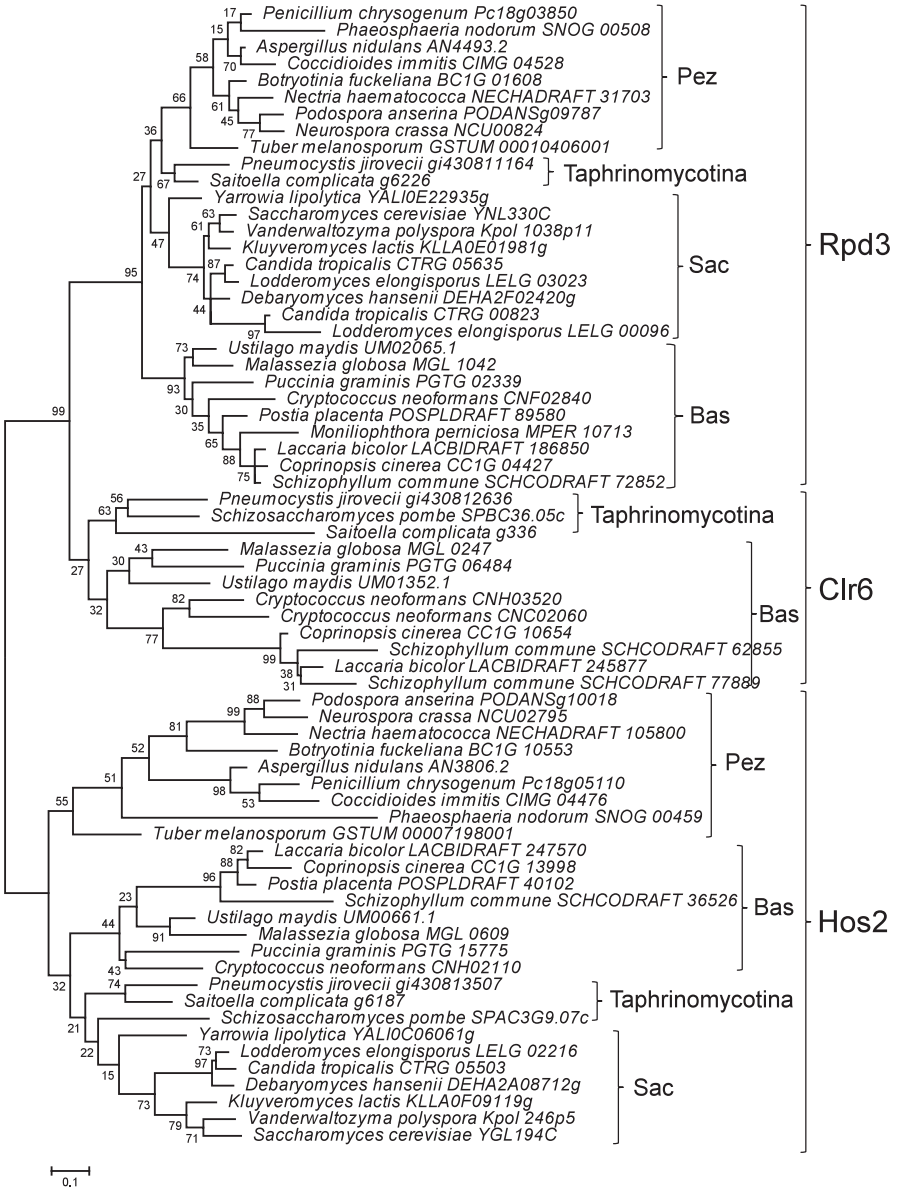

Fig. 2. Phylogenetic relationships among Hos2, Rpd3, and their homologous proteins.

Multiple alignments were generated using MUSCLE (Edgar, 2004). The maximum likelihood tree was reconstructed using MEGA version 5 (Tamura et al., 2011). The general reverse transcriptase model (Dimmic et al., 2002) was used as the amino acid substitution model. The nearest neighbor interchange was used as the maximum likelihood heuristic method. The γ-distributed rate was considered and the number of discrete gamma categories was 5. The bootstrap was performed with 100 replicates. Clr6 of *Schizosaccharomyces pombe* is SPBC36.05c. Hos2 and Rpd3 of *Saccharomyces cerevisiae* are YGL194C and YNL330C, respectively. Bas, Pez, and Sac indicate Basidiomycota, Pezizomycotina, and Saccharomycotina, respectively.

comparative study, because the evolutionary conservation level of these HDACs is the highest among fungal HDACs (Nishida, 2009).

Interestingly, the phylogenetic tree showed that Clr6 of *Schizosaccharomyces pombe* is located at a distance from other ascomycetous Rpd3 proteins and forms a lineage with basidiomycetous proteins, *Pneumocystis jirovecii* gi430812636, and *Saitoella complicata* g336 (Fig. 2). Although the *Schizosaccharomyces* Clr6 has several functional similarities with *Saccharomyces* Rpd3 (Wirén et al., 2005), the phylogenetic tree (Fig. 2) strongly suggests that Clr6-like proteins are functionally different from Rpd3-proteins in Dikarya (ascomycetes and basidiomycetes). Thus, Dikarya have the 3 lineages of Clr6 homologs, Hos2 homologs, and Rpd3 homologs. *Pneumocystis jirovecii* and *Saitoella complicata* are the first ascomycetes found to possess all 3 of these homologs. This suggests that the ancestor of Dikarya had all 3 types of HDACs. During ascomycete evolution, Pezizomycotina and Saccharomycotina appear to have lost their Clr6 homologs and *Schizosaccharomyces* to have lost its Rpd3 homolog.

Acknowledgments

The authors thank Professor Junta Sugiyama for his valuable comments on *Saitoella complicata*. The genome assembly and RNA-seq-based gene prediction were carried out on the supper computer system of Research Organization of Information and Systems (ROIS), National Institute of Genetics (NIG).

References

Altschul, S. F., Gish, W., Miller, W., Meyers, E. W., and Lipman, D. J. (1990) Basic local alignment search tool. *J. Mol. Biol.*, **215**, 403−410.

An, K.-D., Nishida, H., Miura, Y., and Yokota, A. (2002) Aminoadipate reductase: A new fungal-specific gene for comparative evolutionary analyses. *BMC Evol. Biol.*, **2**, 6.

Bjerling, P., Silverstein, R. A., Thon, G., Caudy, A., Grewal, S. et al. (2002) Functional divergence between histone deacetylases in fission yeast by distinct cellular localization and in vivo specificity. *Mol. Cell. Biol.*, **22**, 2170−2181.

Burriesci, M. S., Lehnert, E. M., and Pingle, J. R. (2012) Fulcrum: Condensing redundant reads from high-throughput sequencing studies. *Bioinformatics*, **28**, 1324−1327.

Cissé, O. H., Almeida, J. M., Fonseca, A., Kumar, A. A., Salojärvi, J. et al. (2013) Genome sequencing of the plant pathogen *Taphrina deformans*, the causal agent of peach leaf curl. *mBio*, **4**, e00055−13.

Cisséa, O. H., Pagni, M., and Hauser, P. M. (2012) *De novo* assembly of the *Pneumocystis jirovecii* genome from a single bronchoalveolar lavage fluid specimen from a patient. *mBio*, **4**, e00428−12.

de Ruijter, A.J., van Gennip, A.H., Caron, H.N., Kemp, S., and van Kuilenburg, A.B. (2003) Histone deacetylases (HDACs): Characterization of the classical HDAC family. *Biochem. J.*, **370**, 737−749.

Dimmic, M. W., Rest, J. S., Mindell, D. P., and Goldstein, R. A. (2002) rtREV: An amino acid substitution matrix for inference of retrovirus and reverse transcriptase phylogeny. *J. Mol. Evol.*, **55**, 65−73.

Edgar, R.C. (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.*, **32**, 1792−1797.

Ekwall, K. (2005) Genome-wide analysis of HDAC function. *Trends Genet.*, **21**, 608−615.

Fonseca, Á. and Rodrigues, M. G. (2011) *Taphrina* Fries (1832). The Yeasts, a Taxonomic Study, 5th ed., ed by Kurtzman, C.P., Fell, J.W., and Boekhout, T., Elsevier, Amsterdam, The Netherlands, pp. 823−858.

Goto, S., Sugiyama, J., Hamamoto, M., and Komagata, K. (1987) *Saitoella*, a new anamorph genus in the Cryptococcaceae to accommodate two Himalayan yeast isolates formerly identified as *Rhodotorula glutinis*. *J. Gen. Appl. Microbiol.*, **33**, 75−85.

Igo-Kemenes, T., Hörz, W., and Zachau, H. G. (1982) Chromatin. *Annu. Rev. Biochem.*, **51**, 89−121.

Kent, W. J. (2002) BLAT—the BLAST-like alignment tool. *Genome Res.*, **12**, 656−664.

Kornberg, R. D. (1977) Structure of chromatin. *Annu. Rev. Biochem.*, **46**, 931−954.

Kurtzman, C. P. (2011) *Protomyces* Unger (1833). The Yeasts, a Taxonomic Study, 5th ed., ed. by Kurtzman, C.P., Fell, J.W., and Boekhout, T., Elsevier, Amsterdam, The Netherlands, pp. 725−731.

Kurtzman, C. P. and Robnett, C. J. (2012) *Saitoella coloradoensis* sp. nov., a new species of the Ascomycota, subphylum Taphrinomycotina. *Antonie van Leeuwenhoek*, **101**, 795−802.

Kurtzman, C. P. and Robnett, C. J. (2013) Relationships among genera of the Saccharomycotina (Ascomycota) from multigene phylogenetic analysis of type species. *FEMS Yeast Res.*, **13**, 23−33.

Lee, K. K. and Workman, J. L. (2007) Histone acetyltransferase complexes : One size doesn't fit all. *Nat. Rev. Mol. Cell. Biol.*, **8**, 284−295.

Li, B., Carey, M., and Workman, J. L. (2007) The role of chromatin during transcription. *Cell*, **128**, 707−719.

Li, R., Zhu, H., Ruan, J., Qian, W., Fang, X. et al. (2010) De novo assembly of human genomes with massively parallel short read sequencing. *Genome Res.*, **20**, 265−272.

Liu, Y. J, and Hall, B. D. (2004) Body plan evolution of ascomycetes, as inferred from an RNA polymerase II phylogeny. *Proc. Natl. Acad. Sci. USA*, **101**, 4507−4512.

Liu, Y., Leigh, J. W., Brinkmann, H., Cushion, M. T., Rodriguez-Ezpeleta, N. et al. (2009) Phylogenomic analyses support the monophyly of Taphrinomycotina, including *Schizosaccharomyces* fission yeasts. *Mol. Biol. Evol.*, **26**, 27−34.

Luger, K. and Richmond, T. J. (1998) The histone tails of the nucleosome. *Curr. Opin. Genet. Dev.*, **8**, 140−146.

Lutzoni, F., Kauff, F., Cox, C. J., McLaughlin, D., Celio, G. et al. (2004) Assembling the fungal tree of life: Progress, classification, and evolution of subcellular traits. *Am. J. Bot.*, **91**, 1446−1480.

Nishida, H. (2009) Evolutionary conservation levels of subunits of histone-modifying protein complexes in fungi. *Comp. Funct. Genomics*, **2009**, 379317.

Nishida, H., Hamamoto, M., and Sugiyama, J. (2011) Draft genome sequencing of the enigmatic yeast *Saitoella complicata*. *J. Gen. Appl. Microbiol.*, **57**, 243−246.

Nishida, H. and Sugiyama, J. (1993) Phylogenetic relationships among *Taphrina*, *Saitoella*, and other higher fungi. *Mol. Biol. Evol.*, **10**, 431−436.

Reeb, V., Lutzoni, F., and Roux, C. (2004) Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. *Mol. Phylogenet. Evol.*, **32**, 1036−1060.

Rhind, N., Chen, Z., Yassour, M., Thompson, D. A., Haas, B. J. et al. (2011) Comparative functional genomics of the fission yeasts. *Science*, **332**, 930−936.

Schoch, C. L., Sung, G. H., López-Giráldez, F., Townsend, J. P., Miadlikowska, J. et al. (2009) The Ascomycota tree of life: A phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Syst. Biol.*, **58**, 224−239.

Stanke, M., Diekhans, M., Baertsch, R., and Haussler, D. (2008) Using native and syntenically mapped cDNA alignments to improve de novo gene finding. *Bioinformatics*, **24**, 637−644.

Sugiyama, J., Fukagawa, M., Chiu, S.-W., and Komagata, K. (1985) Cellular carbohydrate composition, DNA base composition, ubiquinone systems, and diazonium blue B color test in the genera *Rhodosporidium*, *Leucosporidium*, *Rhodotorula* and related basidiomycetous yeasts. *J. Gen. Appl. Microbiol.*, **31**, 519−550.

Sugiyama, J. and Hamamoto, M. (2011) *Saitoella* S. Goto, Sugiyama, Hamamoto & Komagata (1987). The Yeasts, a Taxonomic Study, 5th ed., ed. by Kurtzman C.P., Fell J.W., and Boekhout, T., Elsevier, Amsterdam, The Netherlands, pp. 1313−1315.

Sugiyama, J., Hosaka, K., and Suh, S.-O. (2006) Early diverging Ascomycota: Phylogenetic divergence and related evolutionary enigmas. *Mycologia*, **98**, 996−1005.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. et al. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.*, **28**, 2731−2739.

Wang, H., Xu, Z., Gao, L., and Hao, B. (2009) A fungal phylogeny based on 82 complete genomes using the composition vector method. *BMC Evol. Biol.*, **9**, 195.

Wirén, M., Silverstein, R. A., Sinha, I., Walfridsson, J., Lee, H. et al. (2005) Genome wide analysis of nucleosome density histone acetylation and HDAC function in fission yeast. *EMBO J.*, **24**, 2906−2918.

Wood, V., Gwilliam, R., Rajandream, M. A., Lyne, M., Lyne, R. et al. (2002) The genome sequence of *Schizosaccharomyces pombe*. *Nature*, **415**, 871−880.

Yang, X.-J. and Seto, E. (2008) The Rpd3/Hda1 family of lysine deacetylases: From bacteria and yeast to mice and men. *Nat. Rev. Mol. Cell. Biol.*, **9**, 206−218.