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Research article

Aminoadipate reductase gene: a new fungal-specific gene for comparative evolutionary analyses

Kwang-Deuk An, Hiromi Nishida*, Yoshiharu Miura and Akira Yokota

Address: Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

E-mail: Kwang-Deuk An - aa17122@mail.ecc.u-tokyo.ac.jp; Hiromi Nishida* - hnishida@iam.u-tokyo.ac.jp; Yoshiharu Miura - ymiura@iam.u-tokyo.ac.jp; Akira Yokota - uayoko@mail.ecc.u-tokyo.ac.jp

*Corresponding author

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Abstract

Background: In fungi, aminoadipate reductase converts 2-aminoadipate to 2-aminoadipate 6-semialdehyde. However, other organisms have no homologue to the aminoadipate reductase gene and this pathway appears to be restricted to fungi. In this study, we designed degenerate primers for polymerase chain reaction (PCR) amplification of a large fragment of the aminoadipate reductase gene for divergent fungi.

Results: Using these primers, we amplified DNA fragments from the archiascomycetous yeast *Saitoella complicata* and the black-koji mold *Aspergillus awamori*. Based on an alignment of the deduced amino acid sequences, we constructed phylogenetic trees. These trees are consistent with current ascomycete systematics and demonstrate the potential utility of the aminoadipete reductase gene for phylogenetic analyses of fungi.

Conclusions: We believe that the comparison of aminoadipate reductase among species will be useful for molecular ecological and evolutionary studies of fungi, because this enzyme-encoding gene is a fungal-specific gene and generally appears to be single copy.

Background

It is hypothesized that there are 1.5 million fungal species on earth, of which only about 70,000 have been described [1]. Thus nearly 1.43 million remain undescribed. It will be essential for the study of fungal evolution to determine the phylogenetic positions of undescribed fungi present in diverse environments. Many fungi have parasitic or symbiotic relationships to other organisms. This can make it difficult, if not impossible, to separate fungi from such organisms. On the other hand, PCR can be used to amplify the DNA fragments without isolation and cultivation. The genes used most widely in fungal phylogenetic studies are rRNA genes, elongation factor genes, tublin genes, and other universally conserved eukaryotic sequences. These genes are so conserved among all eukaryotes that they often result in artifacts when amplified by PCR. Therefore, we conclude that PCR primers that are unique to fungal genomes will be extremely useful for PCR-based phylogenetic study of fungi.

In addition, although the comparison of rDNA and other genes among species is a powerful tool to show the phylogenetic relationships among fungi, no individual gene can answer all questions about fungal evolutionary relationships [2–4]. Many genes are not useful for quantita-

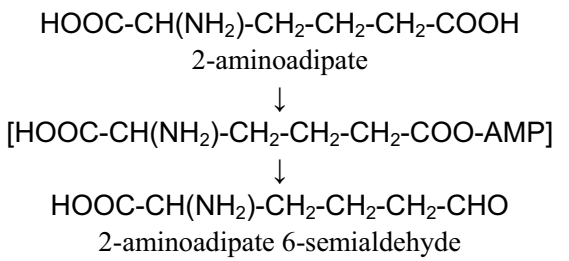

Figure 1
Reduction of 2-aminoadipate by aminoadipte reductase in fungi.

tive analysis because they are multi-copy and copy-number is different among various fungi.

Here we investigate the aminoadipate reductase gene. Most mycologists previously believed that lysine biosynthesis through the 2-aminoadipate pathway was a fungal-specific characteristic. However, some prokaryotes also synthesize lysine through the 2-aminoadipate pathway [5–7]. A comparison between the prokaryotic and fungal lysine biosynthetic pathways reveals that the synthesis of 2-aminoadipate from 2-oxoglutarate proceeds in the same way but the fungal process to synthesize lysine from 2-aminoadipate is different from the prokaryotic one [5,8]. Therefore, prokaryotes have no aminoadipate reductase gene; on the other hand, the aminoadipate reductase is a key enzyme in the evolution of fungal lysine biosynthesis [8]. This enzyme consists of large and small subunits [9], and functions only in fungal cells. As far as we know, animals and plants have no homologue of this enzyme-coding gene.

In the lysine biosynthetic pathway of the ascomycete yeast *Saccharomyces cerevisiae*, a complex of LYS2 (1392 aa) and LYS5 (272 aa) serves as aminoadipate reductase, which converts 2-aminoadipate into 2-aminoadipate 6-semialdehyde via an adenosylated derivative (Fig. 1). Before this report, seven coding regions of the large subunit of the aminoadipate reductase were deposited in the international DNA/protein database [10–15]. The *Saccharomyces cerevisiae lys2* gene is a single-copy gene. Comparisons of single-copy genes of large size can provide good phylogenetic resolution and offer advantages over multi-copy genes [16]. According to the seven ascomycetous aminoadipate reductases, fungal the coding regions of *lys2* genes are more than 4,000 nucleotides-long.

Results and Discussion

First we performed a homology search using BLAST [17] with the given parameter values on the DNA data bank of Japan (DDBJ). We searched the homologous sequence of the *S. cerevisiae lys2* gene for EST database of *Arabidopsis thaliana, Caenorhabditis elegans, Dictyostelium discoideum, Drosophila melanogaster, Glycine max, Homo sapiens, Mus musculus, Oryza sativa, Xenopus laevis*, and *Zea mays*. As a result, we obtained no significant sequence. This result supports that the aminiadipate reductase gene is a fungal-specific gene.

We determined the DNA sequence of 1,058-bp from *S. complicata* and 1,079-bp from *A. awamori*. These sequences have been deposited in the DDBJ under accession numbers AB076076 for *S. complicata* and AB076077 for *A. awamori*. An alignment of the nine ascomycetes was created using the program CLUSTAL W [18]. The alignment in this study is available on request using e-mail. We considered 325 amino acid sites excluding indels and PCR primers-sites. A maximum parsimonious phylogenetic tree was constructed using a Branch-and-Bound algorithm method, in MEGA version 2.1 [19], with 1,000 bootstrap replicates. We obtained equally two most parsimonious trees.

The consensus parsimony tree (Fig. 2a) shows three major ascomycete lineages; the archiascomycete, the euascomycete, and the hemiascomycete. This is consistent with current ascomycete systematics based on other gene sequences. The bootstrap analysis indicated 96% and 90% support values for monophyletic lineages of euascomycetes and hemiascomycetes, respectively. However, it indicated only 57% support for the monophyly of the archiascomycetes.

Maximum likelihood analysis was performed using PAML [20], version 3.1. In this analysis, we used the model of amino acid substitution by Whelan and Goldman [21]. The best tree (lnL: -4292.79) is shown in Fig. 2b. The ML tree is largely consistent with the most parsimonious tree, and shows the archiascomycetes to be monophyletic.

The anamorphic yeast *Saitoella complicata* has unique morphological and chemotaxonomic characteristics, and its 18S rDNA shows affinity to those of archiascomycetes [3,22–24]. As far as we know, this interesting and important yeast has not been analyzed in a molecular evolutionary study, aside from rDNA comparison. Here we determined the DNA fragment from the aminoadipate reductase gene and also show that the deduced amino acid sequence has an affinity for the archiascomycete *Schizosaccharomyces pombe* in phylogenetic analyses. The phylogenetic tree (Figs. 2a, 2b) clearly indicated that the budding yeast *S. complicata* was far from the other budding yeasts (the hemiascomycete lineage).

The phylogenetic position of the black-koji mold *Aspergillus awamori* suggests a close relationship to *Penicillium chrysogenum* (99% bootstrap support). This is an expected

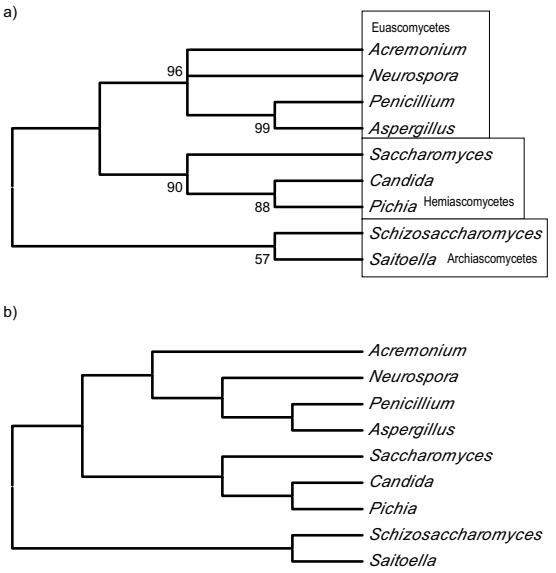

Figure 2
a) The bootstrap consensus tree of the two most parsimonious trees based on the amino acid sequences of the aminoadipate reductase. The most parsimonious trees using the Branch-and-Bound algorithm of MEGA version 2.1 [19] with 1,000 bootstrap analyses. b) The maximum likelihood phylogenetic relationships. This analysis was performed using PAML [20], version 3.1. The model of amino acid substitution by Whelan and Goldman [21] was used.

result. The sequence similarity between *A. awamori* and *P. chrysogenum* is 86% in amino acid comparison and 76% in DNA comparison. This sequence-difference in the aminoadipate reductase comparison is greater than that in shown from rDNA comparison.

Conclusions

The PCR primers designed in this study were shown to be effective for amplifying the aminoadipate reductase gene from divergent ascomycetes. In addition, this region of the PCR product is useful for clarifying the ascomycete phylogeny. We believe that this region would be a powerful tool for fungal ecological and evolutionary studies.

Materials and Methods

In this study we used *Aspergillus awamori* IAM 2112, *Saitoella complicata* IAM 12964. The genomic DNAs were isolated using a DNeasy Plant Mini Kit (QIAGEN, Valencia, CA). Multiple alignment was created among the seven known ascomycetous aminoadipate reductases (*Acremonium chrysogenum*, AJ261064; *Candida albicans*, U58133; *Neurospora crassa*, AL389890; *Penicillium chrysogenum*, Y13967; *Pichia sorbitophila*, AJ288950; *Saccharomyces cerevisiae*, M36287; *Schizosaccharomyces pombe*, AL353014) using the program CLUSTAL W [17]. According to the multiple alignment, we found only two conserved regions for the PCR-primers. Based on the conserved amino acid sequences, two primers were designed 5'-GGNATHGCN-CAYGAYCCNRTNCA-3' and 5'-GGYTTRTCNAYYTTNC-CRTTNGGRTT-3'. The amplification was carried out under the following conditions: denaturation at 94°C for 5 min, 30 cycles of (94°C for 1 min, 57°C for 1 min, 72°C for 1 min), and a final extension at 72°C for 10 min. The PCR products were cloned using a PCR Cloning Kit (QIAGEN). Direct sequencing for the PCR products and sequencing for the several cloned plasmids were performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, CA).

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