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

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 aminoadipate 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-
HOOC-CH(NH₂)-CH₂-CH₂-CH₂-COOH
2-aminoadipate

\[ \text{ZH} \]

[HOOC-CH(NH₂)-CH₂-CH₂-CH₂-COO-AMP]
ZH

HOOC-CH(NH₂)-CH₂-CH₂-CH₂-CHO
2-aminoadipate 6-semialdehyde

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

results 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*, *Glycy Sacramento pombe*, *Homo sapiens*, *Mus musculus*, *Oryza sativa*, *Xenopus laevis*, and *Zea mays.* As a result, we obtained no significant sequence. This result supports that the aminoadipate 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 \textit{A. awamori} and \textit{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 \textit{Aspergillus awamori} IAM 2112, \textit{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 (\textit{Acremonium chrysogenum}, AJ261064; \textit{Candida albicans}, U58133; \textit{Neurospora crassa}, AL389890; \textit{Penicillium chrysogenum}, Y13967; \textit{Pichia sorbitophila}, A1288950; \textit{Saccharomyces cerevisiae}, M36287; \textit{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-CAYGAYCCNRNTNGA-3’ and 5’-GGYTTRTCAYAYYTINC-CRTTNGGRRT-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).

Acknowledgements

We thank the two anonymous reviewers for their helpful comments. The Ministry of Education, Culture, Sports, Science, and Technology of Japan supported this work.

References

1. Hawksworth DL, Rossman AY: Where are all the undescribed fungi? Phytopathology 1997, 87:888-891.
2. Berbee ML, Carmean DA, Winka K: Ribosomal DNA and resolution of branching order among the ascomycota: how many nucleotides are enough? Mol Phylgenet Evol 2000, 17:337-344.
3. Nishida H, Sugiyama J: Phylogenetic relationships among Taphrina, Saitoella, and other higher fungi. Mol. Biol. Evol. 1993, 10:431-436.
4. Nishida H, Sugiyama J: Aschiascomycetes: detection of a major new lineage within the Ascomycota. Mycoscience 1994, 35:361-366.
5. Nishida H, Nishiyama M, Kobashi N, Kosuge T, Hoshino T, Yamane H: A prokaryotic gene cluster involved in synthesis of lysine through the amino adipate pathway: a key to the evolution of amino acid biosynthesis. Genome Res. 1999, 9:1175-1183.
6. Nishida H: Distribution of genes for lysine biosynthesis through the amino adipate pathway among prokaryotic genomes. Bioinformatics 2001, 17:189-191.
7. Schaefer S, Paulme T, Vila R, Fuchs G: 13C-NMR study of acetate assimilation in \textit{Thermoproteus neutrophilus}. Eur. J. Biochem. 1989, 186:695-700.
8. Nishida H, Nishiyama M: What is characteristic of fungal lysine synthesis through the \(\alpha\)-aminoadipate pathway? J. Mol. Evol. 2000, 51:299-302.
9. Zabriskie TM, Jackson MD: Lysine biosynthesis and metabolism in fungi. Nat. Prod. Rep. 2000, 17:85-97.
10. Bleykasten-Grosshans C, Prior C, Potier S: Cloning and sequence of the LYS2 homologue gene from the osmotolerant yeast \textit{Pichia sorbitophila}. Yeast 2001, 18:61-67.
11. Casqueiro J, Gutierrez S, Baneulos O, Fierro F, Velasco J, Martin JF: Characterization of the lys2 gene of \textit{Penicillium chrysogenum} encoding \(\alpha\)-aminoadipic acid reductase. Mol. Gen. Genet 1998, 259:549-556.
12. Ford RA, Bhattacharjee JK: Molecular properties of the lys1+ gene and the regulation of \(\alpha\)-aminoadipate reductase in \textit{Schizosaccharomyces pombe}. Curr. Genet. 1995, 28:131-137.
13. Hijjrabia MJ, Aparicio LF, Casqueiro J, Martin JF: Characterization of the LYS2 gene of \textit{Acremonium chrysogenum} encoding a functional \(\alpha\)-aminoadipate activating and reducing enzyme. Mol. Gen. Genet 2001, 264:755-762.
14. Morris ME, Jinks-Robertson S: Nucleotide sequence of the LYS2 gene of \textit{Saccharomyces cerevisiae} homology to \textit{Bacillus brevis} tyrrocidine synthetase 1. Gene 1991, 98:141-145.
15. Suvarna K, Shah L, Bhattacharjee V, Bhattacharjee JK: Molecular analysis of the LYS2 gene of \textit{Candida albicans} homology to peptide antibiotic synthetases and the regulation of the \(\alpha\)-aminoadipate reductase. Curr. Genet. 1998, 33:268-275.
16. Liu YJ, Whelen S, Hall BD: Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol. Biol. Evol. 1999, 16:1799-1808
17. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: Basic local alignment search tool. J. Mol. Biol 1990, 215:403-410
18. Thompson JD, Higgins DG, Gibson TJ: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994, 22:4673-4680
19. Kumar S, Tamura K, Jakobsen IB, Nei M: MEGA2: Molecular Evolutionary Genetics Analysis software, Arizona State University, Tempe, Arizona, USA 2001
20. Yang Z: PAML: a program package for phylogenetic analysis by maximum likelihood. Comput. Appl. Biosci. 1997, 13:555-556
21. Whelan S, Goldman N: A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. Mol. Biol. Evol. 2001, 18:691-699
22. Goto S, Sugiyama J, Hamamoto M, Komagata K: Saitoella, a new anamorphic genus in the Cryptococcaceae to accommodate two Himalayan yeast isolates formally identified as Rhodotorula glutinis. J. Gen. Appl. Microbiol 1987, 33:75-85
23. Sjamsuridzal W, Tajiri Y, Nishida H, Thuan TB, Kawasaki H, Hirata A, Yokota A, Sugiyama J: Evolutionary relationships of members of the genera Taphrina, Protomyces, Schizosaccharomyces, and related taxa within the archiascomycetes: integrated analysis of genotypic and phenotypic characters. Mycosen 1997, 38:267-280
24. Sugiyama J, Nishida H, Suh S-O: The paradigm of fungal diagnoses and descriptions in the era of molecular systematics: Saitoella complicata as an example. In: The Fungal Holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics 1993, 261-269