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雨生红球藻中 IPP 异构酶基因的系统发育分析

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摘要：通过系统进化树的构建对 IPP 异构酶的系统发育进行分析研究。结果表明，不同来源的 IPP 异构酶基因均是单系分支，并且各个分支有着不同进化模式；似然比分析结果发现，绿藻来源的 IPP 异构酶有 9.8% 的氨基酸位点经受了正选择的压力，其基因的进化模式不同于高等植物和细菌中的 IPP 异构酶基因。

关键词：雨生红球藻；IPP 异构酶基因；系统进化树；似然比分析
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Phylogenetic Analysis of IPP Isomerase Gene in *Haematococcus pluvialis*

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Abstract: By reconstruction of a phylogenetic tree, the phylogeny of IPP isomerase was studied. The results showed that different source IPP isomerase genes form monophyletic clade with 100% bootstrap support, and each clade had different evolution model. 9.8% amino acid sites of IPP isomerase derived from green algal endured positive selection by method of maximum likelihood rate, and the evolutionary pattern of IPP isomerase gene derived from green algal was different from those in higher plants and bacterium.

Key words: *Haematococcus pluvialis*; IPP isomerase gene; Phylogenetic tree; Likelihood ratio test (LRT)

The ketocaronoid astaxanthin (3,3′-dihydroxy-β, β-carotene-4,4′-dione) is produced by a number of microorganisms including the green alga *Haematococcus pluvialis* and the heterobasidiomycetous yeast *Phaffia rhodozyma*[1]. Astaxanthin has been shown to be an extremely efficient antioxidant that provides protection against oxygen free radicals[2-3], acts as an anti-cancer agent[4] and stimulates the immune system[5]. *Haematococcus pluvialis*, which reveals the highest astaxanthin accumulation (up to 4% by dry weight) when exposed to unfavorable growth conditions, seems to be the most suitable microbial source for commercial production of natural astaxanthin[6-8].

The enzyme isopentenyl pyrophosphate (IPP) isomerase catalyzes the revisible isomerization of IPP to produce dimethylallyl pyrophosphate, the initial substrate leading to the biosynthesis of carotenoids and many other long-chain isoprenoids. The IPP isomerase gene (2.3 kb, DQ263307) might play a key role in astaxanthin biosynthesis of *H. pluvialis*. A phylogenetic tree was reconstructed using neighbor-joining method to understand the molecular phylogeny

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of IPP isomerase. In order to test for divergence among different branches in the IPP isomerase phylogeny, maximum likelihood approach and site-branch model were employed to identify specific positively selected sites in the IPP isomerase during the process of evolution.

1 Materials and methods

The deduced amino acid sequences of IPP isomerase gene were compared with other prokaryotes and eukaryotes. The IPP isomerase sequences were obtained from GenBank databases. The GenBank accession numbers of reference IPP isomerases in this study are listed in Fig. 1. A phylogenetic tree was reconstructed by neighbor-joining method[9], as implemented in the program MEGA 2.1[10]. The codeml program of PAML version 3.13[11] was used to estimate d_N/d_S ratioes and identify adaptively evolving sites. The likelihood ratio test (LRT) can compare the branch site model A with the null model A. Codon usage bias is well known to affect estimation of synonymous and nonsynonymous substitution rates[12]. Thus we used two models to account for codon usage bias in all our analyses: F3×4 and F61[11]. The posterior probability that a particular codon site is positively selected can be estimated using the empirical Bayes' approach described by the Software. In order to test for divergence among different branches in the IPP isomerase phylogeny, the branch-site model[12], which allows the ω ratio to vary both among sites and among lineages, was also used to detect molecular adaptation along certain branches.

2 Results

To examine the relationships among different sources of IPP isomerase genes, neighbor-joining method (Fig. 1), maximum parsimony method and maximum likelihood method (data not shown) were used to reconstruct the phylogenetic trees. The subtopologies of the evolutionary trees obtained by these methods are nearly identical and the topologies are reliable by the criteria of bootstrap. Two branches that separate chlorophytes (Hpl and Cre), and higher plants (Cbr, Mal, Zma, and Ath) from other lineages were labeled in Fig. 1. Results obtained form branch-site model indicate that two branches (A and B), representing IPP isomerase from chlorophytes and higher plants, respectively, had undergone adaptive evolution. Moreover, other branches representing IPP isomerase from bacterium had different evolutionary patterns from its counterparts in chlorophytes and higher plants (Fig. 1).

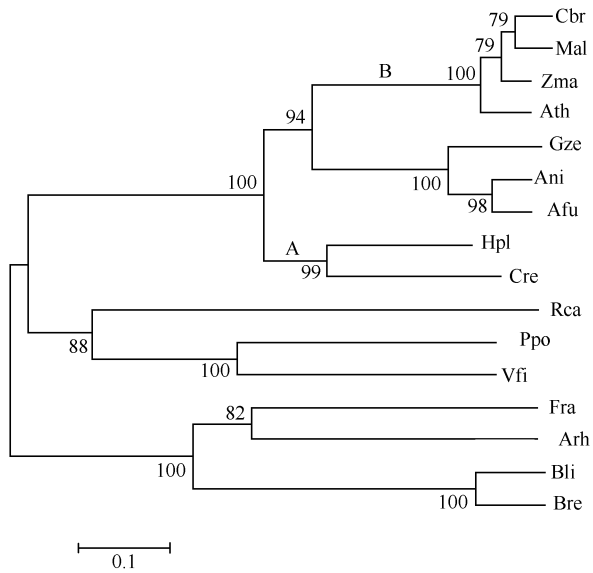

Fig. 1 Phylogenetic trees inferred from IPP isomerase genes using neighbor-joining method

Numbers at the nodes indicate bootstrap values. Scale bars represent level of sequence divergence. Branch A and branch B are used to detect positive selection in later analysis. Hpl = *Haematococcus pluvialis* (BAA33978); Cre = *Chlamydomonas reinhardtii* (AAC32601); Gze = *Gibberella zeae* (EAA77771); Ani = *Aspergillus nidulans* (AAO85433); Afu = *Aspergillus fumigatus* (CAD37150); Cbr = *Clarkia breweri* (CAA57947); Mal = *Melaleuca alternifolia* (AAL91980); Ath = *Arabidopsis thaliana* (BAB09611); Zma = *Zea mays* (AAQ14869); Ppo = *Photobacterium profundum* (YP_128702); Fra = *Frankia* sp. (ZP_00566661); Bre = *Brevibacterium linens* (AAF65591); Vfi = *Vibrio fischeri* (YP_203786); Bli = *Brevibacterium linens* BL2 (ZP_00378967); Rca = *Rhodobacter capsulatus* (CAA77535); Arh = *Agrobacterium rhizogenes* (BAB16172).

The results under two models (null model A and model A) were similar and those under F3×4 were presented only in this paper. Site-specific models: M1a (nearly neutral) vs. M2a (positive selection) were firstly used to estimate d_N/d_S ratioes among sites, but results showed that none of the calculation results (Table 1) suggests the presence of a site with $\omega > 1$.

Results of one-ratio model (M0) gives an estimate $\omega = 0.038$, indicating that IPP isomerase gene family should be under strong selective constraints. All model parameters, likelihood ratio tests and the putative positively selected sites are reported in Table 1. Along branch-A leading to the chlorophytes, 9.8% of sites are under positive selection. This model can be compared with the null model A; the LRT statistic is $2\Delta\ln L = 6.4$, with $P = 0.011$ and d.f. = 1. Moreover, 17% of the codons along branch B are subject to positive selection, with $2\Delta\ln L = 3.94$, and $P = 0.047$. Eight sites (13S, 30S, 53D, 75D, 84F, 86T, 110Q, and 111A, with posterior probability > 0.95) in this lineage were identified to be under positive selection by Bayes empirical Bayes (BEB) method.

Table 1 Parameter estimates for the IPP isomerase gene under site-specific and branch-site models

Model	Parameters	lnL	Positively selected sites
M0: one ratio	$\omega = 0.038$	−6722.24	
M1a: Nearly neutral	$p_0 = 0.981$, ($p_1 = 0.019$)	−6719.82	
	$\omega_0 = 0.038$, $\omega_1 = 1.000$		
M2a: Positive selection	$p_0 = 0.981$, $p_1 = 0.000$, ($p_2 = 0.019$)	−6719.81	None
	$\omega_0 = 0.039$, $\omega_1 = 1.000$, $\omega_2 = 1.000$		
Null Model A			
Branch A	$p_0 = 0.861$, $p_1 = 0.038$,	−6505.40	
	$p_{2a} = 0.097$, $p_{2b} = 0.004$		
	$\omega_0 = 0.036$, $\omega_1 = 1.000$, $\omega_2 = 1.000$		
Branch B	$p_0 = 0.795$, $p_1 = 0.036$,	−6500.51	
	$p_{2a} = 0.161$, $p_{2b} = 0.007$		
	$\omega_0 = 0.035$, $\omega_1 = 1.000$, $\omega_2 = 1.000$		
Model A			
Branch A	$p_0 = 0.862$, $p_1 = 0.039$,	−6502.20	22M 37N 91K 140F ($P > 0.95$)
	$p_{2a} = 0.094$, $p_{2b} = 0.004$		71Q 145C 146N ($P > 0.9$)
	$\omega_0 = 0.039$, $\omega_1 = 1.000$, $\omega_2 = 79.2$		
Branch B	$p_0 = 0.793$, $p_1 = 0.037$,	−6498.54	13S 30S 53D 75D 84F 86T
	$p_{2a} = 0.162$, $p_{2b} = 0.008$		110Q 111A ($P > 0.95$)
	$\omega_0 = 0.037$, $\omega_1 = 1.000$, $\omega_2 = 48.7$		15S 38R 61S ($P > 0.9$)

P is the number of free parameters for the ω distribution. Sites potentially under positive selection are identified using *Haematococcus pluvialis* sequence as the reference.

3 Discussion

IPP isomerase has been found involving in biosynthesis of β-carotene in algae and higher plants. Our work suggested that IPP isomerase was subject to positive selection in the process of evolution. The IPP isomerase gene in chlorophytes exhibits a different evolutionary pattern from its counterparts in higher plants and bacterium. Results showed that 9.8% of sites along IPP isomerase in *H. pluvialis* have undertaken positive selection during evolution. Previous work also showed that PDS (phytoene desaturase), an early enzyme of the carotenoid biosynthesis pathway, which catalyses the conversion of phytoene to ζ-carotene, was also subject to positive selection in the process of evolution. The *pds* in chlorophytes exhibits a different evolutionary pattern from its counterparts in cyanobacteria and higher plants. Results of branch-site model suggested that 7.9% of sites along PDS in *H. pluvialis* be under positive selection[13]. Maybe these amino acid sites result in the different catalyzing activity of IPP isomerase gene in the course of β-carotene accumulation in different organisms. Mutation in these sites and activity assays will help us to determine whether these sites are indispensable to the

catalytic activity and whether the different activities are due to these selected sites in different organisms[14]. Further, sites and genes identified here to have been under positive selection should become interesting targets for functional evaluations.

Although our study on the positively selected sites along IPP isomerase gene is elementary, it provides novel insights into relationships between construction and function of IPP isomerase in *H. pluvialis*.

References

[1] Andrewes A G, Phaff H J, Storr M P. Carotenoids of *Phaffia rhodozyma*, a red-pigmented fermenting yeast [J]. Phytochemistry, 1976, 15: 1003-1005.

[2] Palozza P, Krinsky N I. Astaxanthin and canthaxanthin are potent antioxidants in membrane model [J]. Arch Biochem Biophys, 1992, 297: 291-295.

[3] Miki W. Biological functions and activities of animal carotenoids [J]. Pure Appl Chem, 1991, 63: 141-146.

[4] Tanaka T, Morishita Y, Suzui M, et al. Chemoprevention of mouse urinary bladder carcinogenesis by the naturally occurring carotenoid astaxanthin [J]. Carcinogenesis, 1994, 15: 15-19.

[5] Jyonouchi H, Sun S I, Gross M. Effect of carotenoid on *in vitro* immunoglobulin production by human peripheral blood mononuclear cells: astaxanthin, a carotenoid without vitamin A activity, enhances *in vitro* immunoglobulin production in response to a T-dependent stimulant and antigen [J]. Nutr Cancer, 1995, 23: 171-183.

[6] Kobayashi M, Kakizono T, Nagai S. Enhanced carotenoid biosynthesis by oxidative stress in acetate-induced cyst cells of a green unicellular alga, *Haematococcus pluvialis* [J]. Appl Environ Microbiol, 1993, 59: 867-873.

[7] Boussiba S, Wang B, Yuan P P, et al. Changes in pigments profile in the green alga *Haematococcus pluvialis* exposed to environmental stresses [J]. Biotechn Lett, 1999, 21: 601-604.

[8] Boussiba S. Carotenogenesis in the green alga *Haematococcus pluvialis*: cellular physiology and stress response [J]. Physiol Plant, 2000, 108: 111-117.

[9] Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees [J]. J Mol Evol, 1987, 4: 406-425.

[10] Kumar S, Tamura K, Jakobsen I B. MEGA2: Molecular evolutionary genetics analysis software [J]. Bioinformatics, 2001, 17: 1244-1245.

[11] Yang Z H. PAML: A program package for phylogenetic analysis by maximum likelihood [J]. Cabios, 1997, 13: 555-556.

[12] Yang Z H, Nielsen R. Synonymous and nonsynonymous rate variation in nuclear genes of mammals [J]. J Mol Evol, 1998, 46: 409-418.

[13] Yang Z H, Nielsen R. Codon-substitution models for detecting molecular adaptation at individual sites along specific lineages [J]. Mol Biol Evol, 2002, 19: 908-917.

[14] Liang C W, Zhao F Q, Meng C X, et al. Cloning, characterization and evolutionary analysis of phytoene desaturase (PDS) gene from *Haematoccus pluvialis* [J]. World J Microbiol Biotechn, 2006, 22: 59-64.