Plant Pathology Bulletin 17: 221-232, 2008

Evolution of a histidinol dehydrogenase (*Hdh2*) pseudogene in wheat-biotype *Phaeosphaeria nodorum*

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Accepted for publication: April 7, 2008

ABSTRACT

Wang, C.-L., Lin, Y.-H., Chiu, E. Y. H., and Ueng, P. P. 2008. Evolution of a histidinol dehydrogenase (*Hdh2*) pseudogene in wheat-biotype *Phaeosphaeria nodorum*. Plant Pathol. Bull. 17: 221-232.

Histidinol dehydrogenase (L-histidinol: NAD^+ oxidoreductase, HDH; EC 1.1.1.23) is one of three biosynthetic enzymes encoded by the tri-functional histidine biosynthesis (*his*) gene in high fungi, which catalyzes the last two steps of the histidine biosynthesis. A single histidinol dehydrogenase (*Hdh2*) gene was identified in wheat-biotype *Phaeosphaeria nodorum* (PN-w) and *Phaeosphaeria* sp. from Polish rye (P-rye). The *Hdh2* gene was not present in barley-biotype *P. nodorum* (PN-b), *P. avenaria* f. sp. *avenaria* (Paa), 3 groups of *P. avenaria* f. sp. *triticea* (Pat1, Pat2 and Pat3) and *Phaeosphaeria* sp. from dallis grass (*Paspalum dilatatum* Poir.). Since the *Hdh2* gene in PN-w Sn37-1 isolate was not expressed in the cultures grown in rich and minimal media, it was a pseudo-gene. Most of the amino acids important for substrate binding and Zn^{2+} ligand formation for histidinol dehydrogenase enzymatic activity were well conserved in the Hdh2 protein. In phylogenetic analysis based on the deduced peptide sequences, the Hdh1 peptides in the HIS proteins of the fungi were grouped with the bacteria which had cysteine (C) at both C-116 and C-153 positions in histidinol dehydrogenases. The Hdh2 proteins from 3 *Aspergillus* and 2 *Phaeosphaeria* species were grouped with the bacteria, which had mostly leucine (L) at C-116 and all with valine (V) at C-153 positions in histidinol dehydrogenases. Evolution of the *Hdh2* genes in *Phaeosphaeria* and *Aspergillus* species were discussed.

Key words: *Phaeosphaeria nodorum*, wheat glume blotch, histidinol dehydrogenase, pseudo-gene, phylogenetic analysis

INTRODUCTION

Histidine (His) is one of the essential amino acids that is only synthesized by plants and microorganisms. High histidinol dehydrogenase activity was detected in cattle liver and kidney, and it was suggested that cattle could synthesize sufficient histidine to meet this nutritional requirement [28]. However, histidine is one of the important diet supplements in most of mammals. The origin and evolution of the genes responsible for histidine biosynthesis pathway has been studied in prokaryotes and eukaryotes [5, 6, 12]. In budding yeast (*Saccharomyces cerevisiae* Meyen ex E.C. Hansen), 7 genes of the histidine biosynthetic pathway are scattered in various chromosomes [2]. The *HIS4* gene in this yeast encodes a tri-functional protein, HIS4, which can be divided into three enzymatic activity sub-domains: phosphoribosyl-AMP cyclohydrolase (PRA-CH; EC 3.5.4.19), phosphoribosyl-ATP pyrophosphohydrolase (PRA-PH; EC 3.6.1.31) and histidinol dehydrogenase (L-histidinol: NAD^+ oxidoreductase, HDH; EC 1.1.1.23), catalyzes the third, the second and the last two steps in histidine biosynthesis, respectively [9]. In bacteria, fission yeast (*Schizosaccharomyces pombe* Lindner), and cabbage plant (*Brassica oleracea* L.), HDH is present as a single peptide, whereas PRA-CH and PRA-PH are combined in a single bifunctional peptide [3, 14, 19]. Like other enzymes of the histidine synthesis pathway, the sequences of histidinol dehydrogenase (HisD) in bacteria and tri-functional histidine biosynthesis protein (HIS4) in yeasts have been well conserved during evolution [2].

Genetic diversity of the tri-functional histidine biosynthesis (*his*) gene in *Phaeosphaeria* pathogens, causing leaf and glume blotches in cereals, has been recently studied [20, 29]. In addition to the 3rd sub-domain with histidinol dehydrogenase enzymatic activity (HDH, called Hdh1 here) in the HIS protein (SNOG_11402, accession nos. EAT81110), a less similar (ca 30%) 447 aa size polypeptide (SNOG_07871; accession no. EAT85337) was picked in a blast search of the Australian *P. nodorum* isolate SN15 genome sequencing (www.broad.mit.edu). Along with three single deduced hypothetical proteins (ca. 68-74% in similarity) found in three different *Aspergillus* species (*A. fumigatus*-accession no. XP_753132, *A. nidulans*-accession no. XP_660327 and *A. oryzae*-accession no. BAE55256), this HDH polypeptide in PN-w, called *Hdh2* here, was similar to numerous prokaryote HDH proteins, such as 63% for *Corynebacterium glutamicum* (accession no. BAD83947) and 50% for *Rhizobium* sp. (accession no. AAQ87366). It is interesting that there are two potentially encoded histidinol dehydrogenase (*Hdh*) genes present in the genome of a fungal pathogen, such as *P. nodorum*. In order to study its evolutionary significance in the histidine biosynthesis pathway, presence and expression of this *Hdh2* gene in cereal *Phaeosphaeria* species was investigated.

MATERIALS AND METHODS

Hdh2 gene amplifications and sequencings

Procedures for fungal culture in a liquid medium and for genomic DNA (gDNA) isolation were described previously [26]. To study the genetic diversities in PN-w, two primer sets, 21A/21B (ATGCCTCCTAGATATCTAAAGAAAGG/CAGCAGTTCGTCAATGAACGCTAT) and 22A/22B (GCTTTCGTAGCAGAAGGTAAAAGACA/CTAGATCATCGCCCTTTTTGTCAC), designed from the *Hdh2* gene genomic sequence of PN-w isolate SN15 (accession no. CH445335.1: nt1265892 - nt1267387) were used for amplifying the same gene in other 27 PN-w isolates and 2 *Phaeosphaeria* sp. from Polish rye (P-rye) [21] (Table 1 and Fig. 1). These primer sets produced overlapping fragments that together represented the full-length *Hdh2* gene-coding region. The PCR products were resolved by 0.8% agarose gel electrophoresis and eluted from agarose gel blocks with a unidirectional electroelutor [27]. In addition to two primer sets (21A, 21B, 22A, and 22B), 23A (CTGTTCTTGGGTGAGAACACC) and 23B (GACGGCTTGAATACCACCAAG) primers were used to complete the nucleotide sequencings (Fig. 1). Direct sequencing of PCR products was conducted as described previously [27].

Southern blot and gene expression

Ten μg of gDNA from cereal *Phaeosphaeria* species including 3 isolates of each PN-w, barley-biotype *P. nodorum* (PN-b), *P. avenaria* f. sp. *avenaria* (Paa), and homothallic *P. avenaria* f. sp. *triticea* (*P. a.* f. sp. *t.*, Pat1), 2 of each heterothallic *P. a.* f. sp. *t.* (Pat2) and *Phaeosphaeria* sp. from Polish rye (P-rye) [21] and 1 of each

Table 1. Isolates of cereal *Phaeosphaeria* species used for analysis of the pseudo histidinol dehydrogenase (*hdh2*) gene

Species	Original host	Year	Geographic location	GenBank accession number
Phaeosphaeria nodorum (E. Müll.) Hedjar. (wheat-biotype) (PN-w)				
9074	Wheat (*Triticum aestivum* L.)	1983	Gallatin County, MT, USA	EU267782
9076	Wheat	1986	Richland County, MT, USA	(= EU267782)
8408	Wheat	1986	Mandan, ND, USA	(= EU267782)
98-12981	Rye (*Secale cereale* L.)	1998	Mandan, ND, USA	(= EU267782)
9506	Wheat	1987	Mandan, ND, USA	EU267783
Sn26-1	Wheat	-	Rzeszów, Poland	(= EU267783)
Sn27-1	Wheat	-	Sieradz, Poland	(= EU267783)
S-74-20A (ATCC200806)	Wheat	1975	Griffin, GA, USA	(= EU267783)
S-81-W13	Wheat	1981	Marion County, OR, USA	(= EU267783)
S-81-W15	Wheat	1981	Sheridan, OR, USA	(= EU267783)
S-81-W16	Wheat	1981	Harrisburg, OR, USA	(= EU267783)
406	Wheat	1994	New York, USA	(= EU267783)
407	Wheat	1994	New York, USA	(= EU267783)
IN39	Wheat	1993	Indiana, USA	(= EU267783)
IN43	Wheat	1993	Indiana, USA	(= EU267783)
IN46	Wheat	1993	Indiana, USA	(= EU267783)
Sn37-1	Wheat	-	Szelejewo, Poland	EU267784
S-78-13	Wheat	1978	Toluca, Mexico	(= EU267784)
409	Wheat	1994	New York, USA	(= EU267784)
S-80-301	Triticale (×*Triticosecale*)	1980	Williamson, GA, USA	-
S-81-B13B	Barley (*Hordeum vulgare* L.)	1981	Bledsoe, GA, USA	EU267785
S-81-W12	Wheat	1981	Marion County, OR, USA	EU267786
411	Wheat	1994	New York, USA	(= EU267786)
S-87-2	Wheat ('Oasis')	1987	Griffin, GA, USA	EU267787
414	Wheat	1994	New York, USA	(= EU267787)
IN15	Wheat	1993	Indiana, USA	(= EU267787)
IN38	Wheat	1993	Indiana, USA	(= EU267787)
Phaeosphaeria sp. (From Poland) (P-rye)				
Sn48-1	Winter rye	1995	Jelenia Góra, Poland	EU267788
Sn23-1	Winter rye	-	Bydgoszcz, Poland	(= EU267788)
Phaeosphaeria nodorum (E. Müll.) Hedjar. (barley-biotype) (PN-b)				
S-82-13 (ATCC200805)	Barley	1982	Senoia, GA, USA	-
S-83-7	Barley	1983	Holland, VA, USA	-
S-92-7	Barley	1992	Raleigh, NC, USA	-
Phaeosphaeria avenaria f. sp. *avenaria* (Weber) O.E. Erikss. (Paa)				
1920WRS	Oat	2002	Manitoba, Canada	-
ATCC12277	Oat	-	USA	-
Sa37-2	Oat	2001	Radzików, Poland	-
Phaeosphaeria avenaria f. sp. *triticea* (T. Johnson) Shoemaker & C.E. Babc. (Pat1)				
Sat24-1	Wheat	-	Warmińsko-Mazurskie, Poland	-
ATCC26374	Foxtail barley (*Hordeum jubatum* L.)	1972	Minnesota, USA	-
ATCC26375	Foxtail barley	1972	Minnesota, USA	-
Phaeosphaeria avenaria f. sp. *triticea* (T. Johnson) Shoemaker & C.E. Babc. (Pat2)				
ATCC26370	Foxtail barley	1972	Minnesota, USA	-
ATCC26377	Foxtail barley	1972	Minnesota, USA	-
Phaeosphaeria avenaria f. sp. *triticea* (T. Johnson) Shoemaker & C.E. Babc. (Pat3)				
S-81-W10	Wheat	1981	Washington, USA	-
Phaeosphaeria sp. (P-dg)				
S-93-48	Dallis grass (*Paspalum dilatatum* Poir.)	1993	Griffin, GA, USA	-

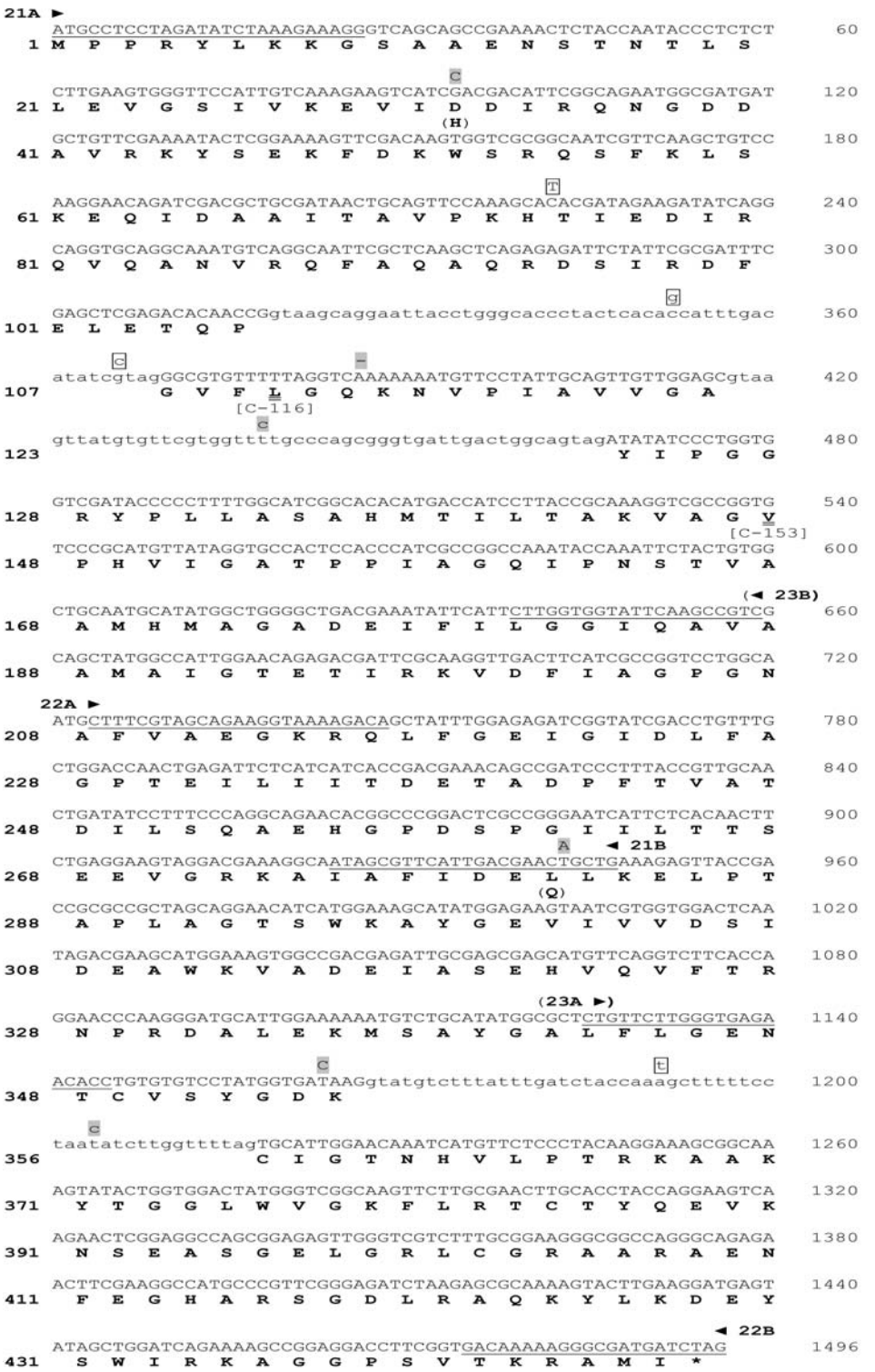

Fig. 1. Nucleotide and deduced amino acid sequences of the histidinol dehydrogenase (*Hdh2*) gene in wheat-biotype *Phaeosphaeria nodorum* isolate 9506. The numerical numbers on the right column were for nucleotides and those on the left for amino acids. Sequences for two primer sets (21A/21B and 22A/22B) used for the *Hdh2* gene amplification and sequencings, and for two primers (23A and 23B) in parentheses for sequencings were single-underlined. Three intron sequences were in lowercase letters whilst four exons in uppercase letters. Nucleotide substitutions occurred in the *Hdh2* gene of other wheat-biotype *P. nodorum* isolates were shaded and indicated above the nucleotides which they replaced. Four nucleotide mutations found in the *Hdh2* gene of two *Phaeosphaeria* sp. isolates (Sn23-1 and Sn48-1) from Polish rye were boxed. The C-116 and C-153 positions (bracketed) corresponding to the amino acid positions in histidinol dehydrogenase (Hdh1) protein of *Salmonella typhimurium* (accession no. AAL20976) were double-underlined.

P. a. f. sp. *t.* from the state of Washington (Pat3) and *Phaeosphaeria* sp. from dallis grass (*Paspalum dilatatum* Poir.) (P-dg grass) were restricted with 60 units of *Bam*H1 endonuclease enzyme (Promega, Madison, WI) for 6 h at 37 ℃ (Table 1, Fig. 2). The digested gDNA fragments were separated with 0.7% agarose gel electrophoresis and transferred to a GeneScreen Plus hybridization transfer membrane (Perkin Elmer, Waltham, Massachusetts, USA) [22]. The primer set 21A/21B amplified *Hdh2* gene fragment was α-[^{32}P]-dATP labeled using the Prime-a-Gene® Labelling System (Promega, Madison, WI), and used as a probe for hybridization. After hybridization, the membrane was washed under high stringency conditions (twice in 2 ×SSC, 0.1% SDS at 65℃ for 30 min; twice in 0.5 ×SSC, 0.1% SDS at 65℃ for 30 min and twice in 0.1 ×SSC, 0.1% SDS at 65℃ for 30 min) and subjected to autoradiography.

To determine the *Hdh2* gene expression in PN-w, total RNA was isolated from the Sn37-1 cultures grown in either minimal (30 g sucrose, 2 g NaNO3, 1 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01g $ZnSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$ and 0.0025 g $CuSO_4 \cdot 5H_2O$ per liter) [23] or YMS rich (0.5% malt extract, 0.5% yeast extract and 2.0% sucrose) [26] liquid media with shaking at 125 rpm for 7-14 days at 27℃. Mycelia were harvested, washed with 2% NaCl solution and flash-frozen in liquid nitrogen. The total RNA was extracted from mortar/pestle pulverized mycelia using the RNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) and RNase-Free DNase I enzyme (Roche Diagnostics Corporation, Indianapolis, IN). Using the First Strand cDNA Synthesis Kit with the Oligo-p$(dT)_{15}$ primer (Roche Diagnostics Corporation, Indianapolis, IN) and the thermocycler settings (25℃ for 10 min, 42℃ for 60 min, 99℃ for 5 min, and 4℃ for 5 min), the first strand cDNA (1 ×cDNA) synthesis was performed. Expression of the *his* gene was used as control. To determine the *Hdh2* and *his* gene transcripts in 1 ×cDNA, primer sets 21A/21B and 15A/12-1 (ATGCCGGCAGGACCCAGTGA/CTATCAAGCTACGCCAAGTCGC) (Fig. 1) [28], were used to amplify the 1 ×cDNA, respectively, with the Advantage cDNA PCR Kit (BD Biosciences-Clontech, Mountain View, CA). Reaction parameters were: 94℃ for 1 min, 34 cycles of 94℃ for 30 s, 55℃ for 3 min and 68℃ for 3 min.

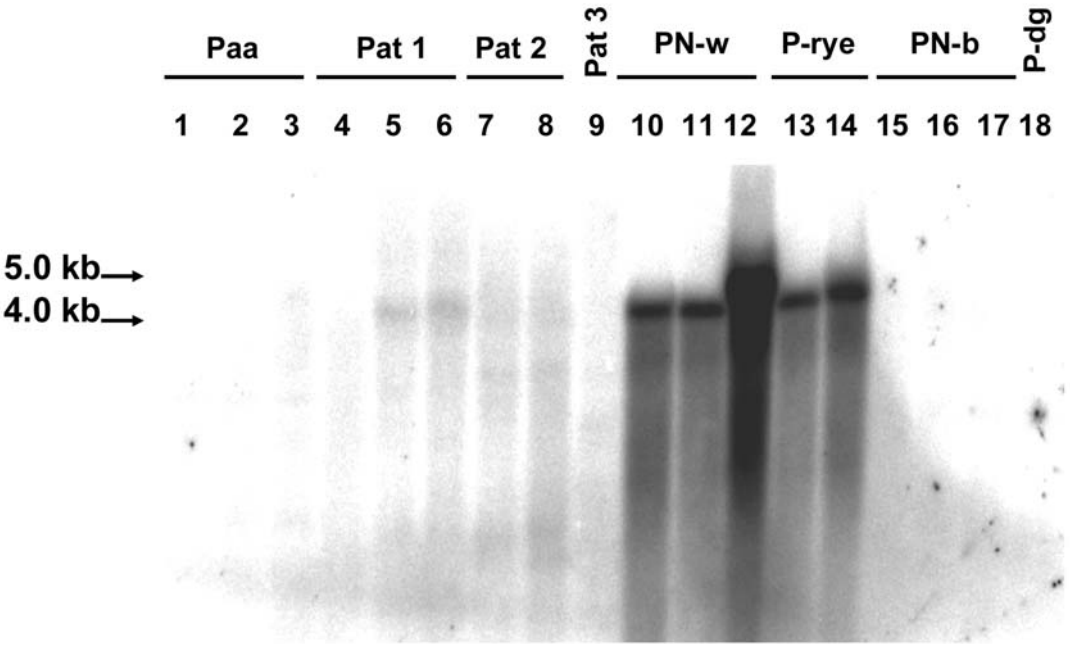

Fig. 2. Southern blot DNA hybridization of the histidinol dehydrogenase (*Hdh2*) gene in *Phaeosphaeria* species. 1 - 3 = *P. avenaria* f. sp. *avenaria* (Paa) isolates ATCC12277, Sa37-1 and 1920WRS; 4 - 6 = homothallic *P. a.* f. sp. *triticea* (Pat1) isolates Sat24-1, ATCC26374 and ATCC26375; 7 - 8 = heterothallic *P. a.* f. sp. *triticea* (Pat2) isolates ATCC26370 and ATCC26377; 9 = *P. a.* f. sp. *triticea* (Pat3) isolate S-81-W10; 10 - 12 = wheat-biotype *P. nodorum* (PN-w) isolate S-74-20A, Sn37-1 and Sn26-1; 13 - 14 = *Phaeosphaeria* sp. (P-rye) isolates Sn48-1 and Sn23-1 from Polish rye; 15 - 17 = barley-biotype *P. nodorum* (PN-b) isolates S-82-13, S-83-7 and S-92-7; 18 = *Phaeosphaeria* sp. isolate S-93-48 from dallis grass (P-dg).

Phylogenetic analysis

Amino acid sequences similar to HDH were identified by protein-protein BLAST search with histidinol dehydrogenase (Hdh1) amino acid sequence of the tri-functional histidine biosynthesis (His) polypeptide (Accession no. EAT81110) and the single Hdh2 polypeptide sequence (EAT85337) of PN-w SN15 isolate from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST/), Fungal Genome Initiative of Broad Institute (http://www.broad.mit.edu/annotation/fgi/) and Munich information center for protein sequences (http://pedant.gsf.de). Deduced histidinol dehydrogenase (HDH) protein sequences of bacteria, fission yeast (*Schizosaccharomyces pombe*), and 11 fungi including 1 Zygomycete, 3 Basidiomycetes and 7 Ascomycetes, and the Hdh2 protein sequences from PN-w and 3 *Aspergillus* spp. were used for phylogenetic analysis. The polypeptide sequences ranging from 408 to 448 amino acids were aligned with CLUSTAL_ ×(1.83) in a multiple sequence alignment mode[25]. From the aligned sequences, 500 data sets were generated by bootstrap re-sampling in the "seqboot" program of Phylogeny Inference Package (PHYLIP) Version 3.66 (http://evolution.genetics.washington.edu/phylip.html)[13]. The bootstrapped data sets were evaluated by the maximum likelihood (ML) method using the "proml" program. Finally the "consense" program was used to construct a TREE.

RESULTS AND DISCUSSION

Four exons and three introns (nt319 - nt369, nt417 - nt466 and nt1167 - nt1217 in Fig. 1) were predicted in the 1,496-bp size *Hdh2* gene in PN-w and P-rye using the FGENESH program (http://www.softberry.com) with *Aspergillus* as the organism parameter (Fig. 1). The *Hdh2* gene coding sequences amplified from PN-w and P-rye had the same length with various nucleotide substitutions (Fig. 1). Based on the *Hdh2* gene genomic sequence of PN-w isolate SN15 (accession no. CH445335; nt1265892 - 1267387), 13 PN-w isolates inculding 9506 had the same nucleotide sequence (accession no. EU267783). Nucleotide substitutions occurred in other PN-w isolates at nt94 (G → C) in 9074/9076/98-12981/8408 (accession no. EU267782), at nt392 (missing A) in S-80-301, at nt438 (t → c) in 411/S-81-W12 (accession no. EU267786), at nt943 (T → A) in S-81-B13B (accession no. EU267785), at nt1163 (T → C) in 409/Sn37-1/S-78-13 (accession no. EU267784) and at nt1163 (T → C) and nt1204 (t → c) in IN15/IN38/414/S-87-2 (accession no. EU267787). (Note: lowercase letters indicated the nucleotide substitutions occurred in the intron regions). Four substitutions at different positions of the *Hdh2* gene, nt222 (C → T), nt353 (c → g), nt366 (g → c) and nt1191 (a → t), were found in two P-rye isolates, Sn48-1 and Sn23-1 (accession no. 267788) (Fig. 1, Table 1).

As deduced from nucleotide sequencings, the 1,344 bp exon sequence in the *Hdh2* gene encoded a 447 aa Hdh2 protein in PN-w and P-rye, except in S-80-301. Theoretically, missing of 'A' nucleotide at nt392 shifted the reading frame in the transcript and abolished the translation of the gene. Since most of nucleotide differences occurred either in introns (Fig. 1) or in exons at the third codons of amino acid coding triplets, they did not affect the amino acid composition of Hdh2 protein in PN-w (accession no. ABX60498) and P-rye (accession no. ABX60503), with the exception of two groups of PN-w isolates. The first codon substitution (G to C) in an aspartic acid (D) triplet at nt94 changed the aa32 to histidine (H) (accession no. ABX60497), and the second substitution (T to A) in a leucine (L) triplet at nt943 became glutamine (Q) at aa281 (accession no. ABX60500) (Fig. 1).

The primer sets (21A/21B and 22A/22B) designing to amplify the *Hdh2* gene from PN-w and P-rye could not isolate the same gene from the other *Phaeosphaeria* species. No PCR fragments could be produced from the gDNA in PN-b, Paa, P-dg and 3 Pat groups (Pat1, Pat2 and Pat3) (Ueng, unpublished data). In Southern blot hybridization, strong radioactive signals as a definite band were seen in PN-w and P-rye isolates (Fig. 2). It appeared that the *Hdh2* gene was only present in PN-w and P-rye isolates.

Also, the *Hdh2* gene in PN-w isolate Sn37-1 was a pseudo-gene since no PCR product could be amplified from 1 × cDNA (Fig. 3). Unlike the *Hdh1* gene fragment in the tri-functional histidine biosynthesis (*his*) gene, the *Hdh2* gene was not expressed in the cultures grown in minimal and rich media (Fig. 3). In a search of noncoding region sequence upstream of the *Hdh2* coding gene, no transcription factor binding element similar to the binding site for general regulation of amino acid, such as

'ACGACTCAT' of the *his* gene in PN-w [29], 'ATGACTCAT' of the *cpc-1* gene in *Neurospora crassa* [10] and 'TGACTC' of the *HIS4* gene in *Saccharomyces cerevisiae* [18] was detected (Ueng, unpublished data).

The Hdh1 sub domain of the tri-functional His polypeptide in PN-w and other ascomycetes had structures homologous to the proteins encoded by the *hisD* genes of *Escherichia coli* (accession no. AAC75081) and *Salmonella typhimurium* (accession no. AAL20976) [4, 7, 9, 16, 30]. However, most of the amino acids important for substrate binding and Zn^{2+} ligand formation for HDH enzymatic activity were also well conserved in the Hdh2 protein, except that the cysteine (C) at aa116 and aa153 in *S. typhimurium* HDH polypeptide. Cysteine (aa561 and aa598) and lysine residues (aa666), corresponding to C-116, C-153 and K-218 in *S. typhimurium* HDH and contributing to formation of a thiohemiacetal intermediate in the first step of bi-functional reaction, were conserved in PN-w Hdh1 sub domain [1, 8, 15, 17]. However, the presence of cysteine (C) in the first two positions (C-116 and C-153) was reported not to be essential. Substitutions with either alanine (A) or/and serine (S) at these two C-116 and C-153 positions in the HDH proteins from *S. typhimurium* functioned normally but with less stability and high sensitivity to chelating agent [24]. It was suggested that cysteine (C)-based thiohemiacetal was not necessarily a catalytic intermediate in HDH enzymatic activity. Other alternative routes for histidine synthesis, such as hydration of aldehydes, were also proposed to be functional [11].

In a previous phylogenetic study of histidinol dehydrogenase (HisD) proteins in bacteria, several species (called Proteobacteria B) were grouped with Hdh1 peptides from eukaryotic fungi [5]. Like the Hdh1 amino acid sequences in eukaryotic fungi, both C-116 and C-153 positions in proteobacteria B and those bacteria grouped in the Clade I in this study were cysteine (C) (Fig. 4). On the other hand, the amino acids at these two positions were different in proteobacteria A [5] and the bacteria species

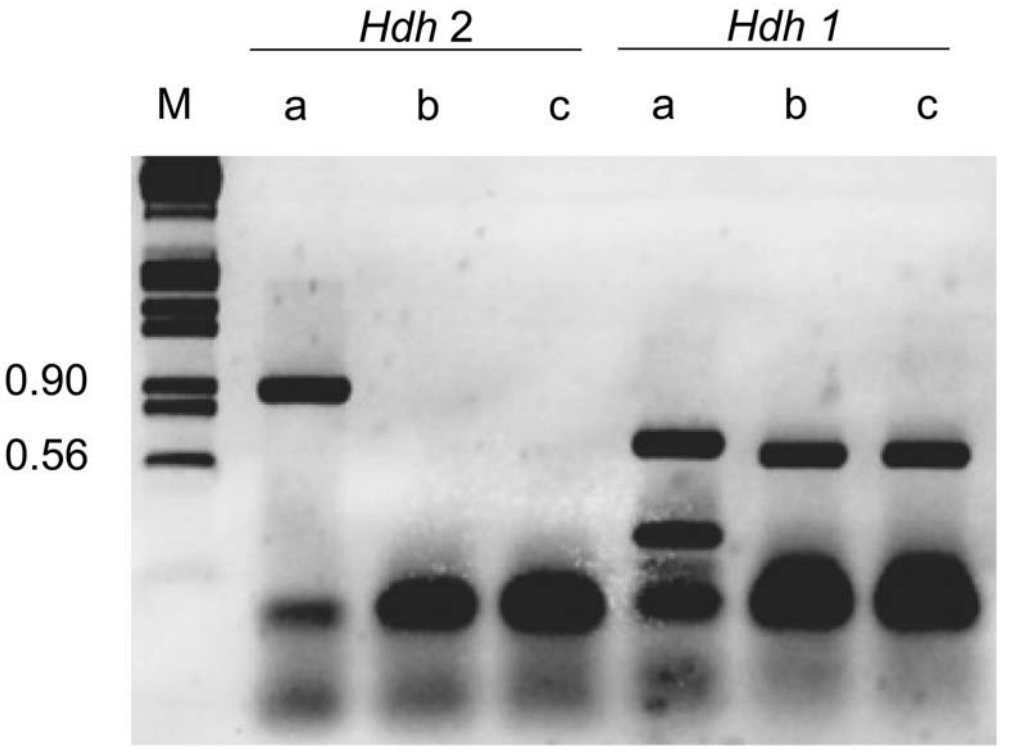

Fig. 3. Expression of two histidinol dehydrogenase, *Hdh1* and *Hdh2*, genes in wheat-biotype *Phaeosphaeria nodorum* (PN-w). Primer sets 21A /21B (ATGCCTCCTAGATATCTAAAGAAAGG / CAGCAGTTCGTCAATGAACGCTAT) and 15A / 12-1 (ATGCCGGCAGGACCCAGTGA / CTATCAAGCTACGCCAAGTCGC) were used to amplify the single *Hdh2* gene and the nucleotide sequence encoding the histidinol dehydrogenase (*Hdh1*) polypeptide in the tri-functional histidine biosynthesis (*his*) gene in PN-w isolate Sn37-1, respectively. Genomic DNA (a) and first strand cDNA from cultures grown in rich medium (b) and minimal medium (c) were used as templates. M = *Hin*dIII/*Eco*RI-digested λ DNA used as molecular markers (size in base pairs).

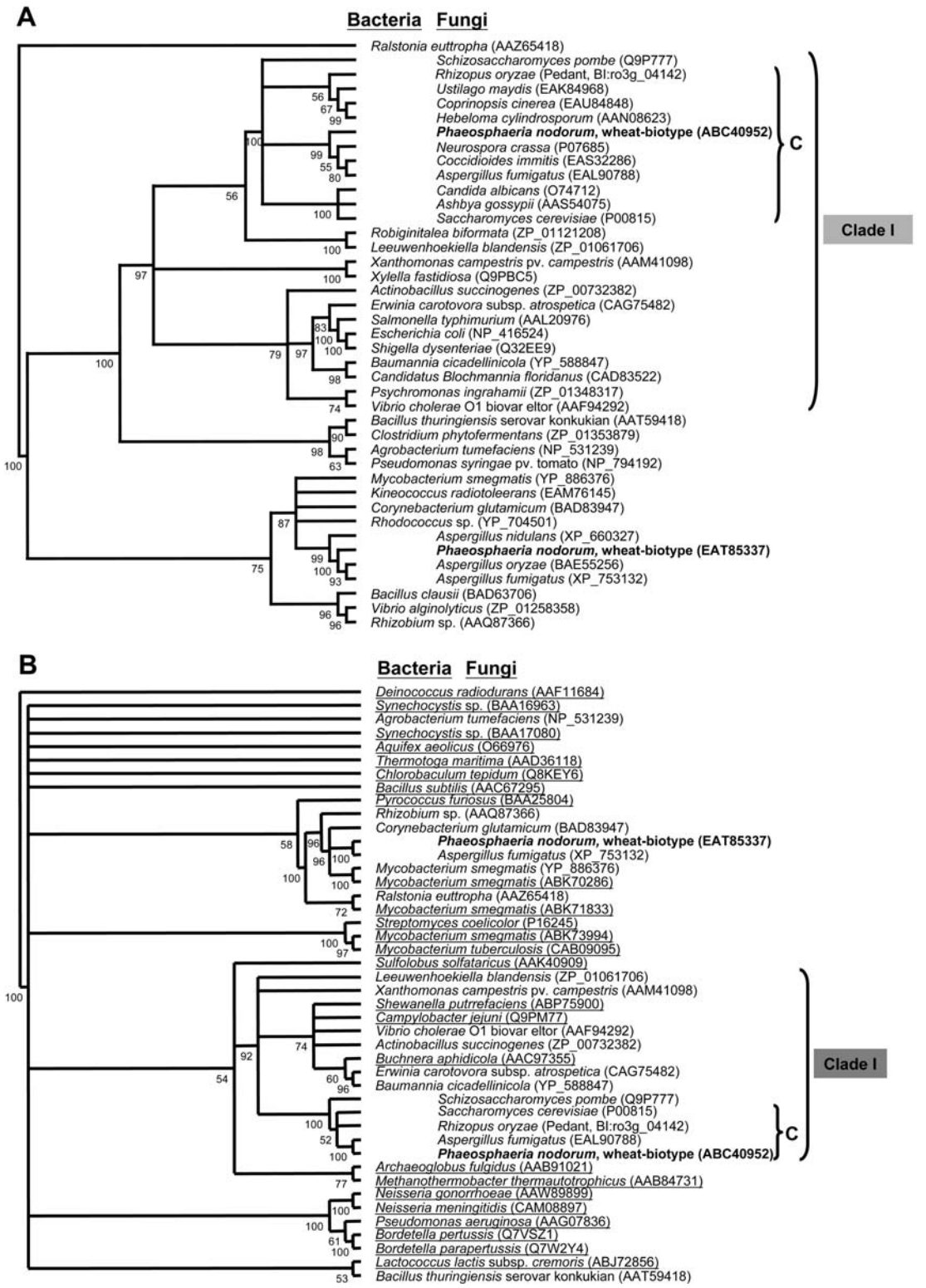

Fig. 4. Phylogenetic relationships based on the deduced amino acid sequences of the histidinol dehydrogenase (Hdh1 and Hdh2) proteins. GenBank accession numbers and a Pedant 3 data bases number (http://pedant.gsf.de) were in parentheses. 25 Hdh1 proteins were grouped as the "Clade I", and the other 16 Hdh2 proteins were outside of the Clade I in (A). Bacterial histidinol dehydrogenase (HDH) protein sequences used by Bond and Francklyn (2000)[5] for phylogenetical study were included in this study and underlined in (B). 13 Hdh1 proteins were grouped as the "Clade I" (B). 11 Hdh1 in (A) and 4 in (B) proteins (indicated as 'C') are part of the polypeptides encoded by the tri-functional histidine biosynthesis (*his*) gene in fungi. Bootstrap values with 500 replications of the internal branches are indicated. Hdh1 and Hdh2 proteins in wheat-biotype *Phaeosphaeria nodorum* were bold.

Table 2. Amino acid substitutions at the positions C-116 and C-153 of histidinol dehydrogenase proteins in bacteria and fungi

Species	Amino acids	
	Position C-116 [a]	Position C-153 [a]
Inside of Clade I group [b]		
Hdh1 peptides of the HIS proteins in fungi	Cysteine	Cysteine
Hdh proteins in bacteria	Cysteine	Cysteine
Outside of Clade I group [b]		
Hdh2 proteins in fungi		
Aspergillus fumigatus (XP_753132)	Leucine	Valine
Aspergillus nidulans (XP_660327)	Leucine	Valine
Aspergillus oryzae (BAE55256)	Leucine	Valine
Phaeosphaeria nodorum, wheat-biotype (ABX60498)	Leucine	Valine
Phaeosphaeria sp. (from Polish rye) (ABX60503)	Leucine	Valine
HDH proteins in bacteria		
Agrobacterium tumefaciens (NP_531239)	Leucine	Valine
Bacillus clausii (BAD63706)	Leucine	Valine
Clostridium phytofermentans (ZP_01353879)	Leucine	Valine
Kineococcus radiotoleerans (EAM76145)	Leucine	Valine
Mycobacterium smegmatis (YP_886376)	Leucine	Valine
Pseudomonas syringae pv. tomato (NP_794192)	Leucine	Valine
Rhizobium sp. (AAQ87366)	Leucine	Valine
Rhodococcus sp. (YP_704501)	Leucine	Valine
Vibrio alginolyticus (ZP_01258358)	Leucine	Valine
Aquifex aeolicus (O66976) [c]	Leucine	Valine
Bacillu subtilis (AAC67295)	Leucine	Valine
Bordetella pertussis (Q7VSZ1)	Leucine	Valine
Chlorobaculum tepidum (Q8KEY6)	Leucine	Valine
Deinococcus radiodurans (AAF11684)	Leucine	Valine
Neisseria gonorrhoeae (AAW89899)	Leucine	Valine
Neisseria meningitidis (CAM08897)	Leucine	Valine
Mycobacterium smegmatis (ABK70286)	Leucine	Valine
Pseudomonas aeruginosa (AAG07836)	Leucine	Valine
Thermotoga maritima (AAD36118)	Leucine	Valine
Ralstonia euttropha (AAZ65418)	Alanine	Valine
Methanothermobacter thermautotrophicus (AAB84731) [d]	Alanine	Valine
Synechocystis sp. (BAA17080)	Alanine	Valine
Bacillus thuringiensis serovar konkukian (AAT59418)	Arginine	Valine
Synechocystis sp. (BAA16963)	Arginine	Valine
Mycobacterium smegmatis (ABK71833)	Glycine	Valine
Corynebacterium glutamicum (BAD83947)	Isoleucine	Valine
Clostridium acetobutylicum (AAK78913)	Methionine	Valine
Archaeoglobus fulgidus (AAB91021) [d]	Methionine	Valine
Lactococcus lactis subsp. *cremoris* (ABJ72856)	Methionine	Valine
Sulfolobus solfataricus (AAK40909)	Phenylalanine	Valine
Pyrococcus furiosus (BAA25804)	Tyrosine	Valine
Staphylococcus aureus subsp. *aureus* (YP_187488)	Tyrosine	Valine
Mycobacterium tuberculosis (CAB09095)	Valine	Valine
Streptomyces coelicolar (CAA45767)	Valine	Valine

[a] The C-116 and C-153 positions in histidinol dehydrogenase proteins were determined by aligning the proteins (HDH in bacteria, and Hdh1 and Hdh2 in fungi) with the histidinol dehydrogenase protein (hisD) sequence in *Escherichia coli* (accession number NP_416524).

[b] For species included in phylogenetic analysis of histidinol dehydrogenase proteins, see Fig 4. Ninteen proteobacteria A.

[c] and two archaea

[d] used for phylogenetic analysis of histidinol dehydrogenase protein sequences (Hdh) by Bond and Francklyn (2000) [5] were underlined.

outside of the Clade I (Fig. 4A). All bacteria outside of the Clade I and 5 fungi including 3 *Aspergillus* and 2 *Phaeosphaeria* species had valine (V) at the C-153 position, and mostly with leucine (L) at the C-116 position in the Hdh2 proteins (Table 2). One exception was an Archaea species, *Methanocaldococcus* (*Methanococcus*) *jannaschii* (accession no. AAB99465), which had leucine (L) and cysteine (C) at the positions C-116 and C-153, respectively (Ueng, unpublished data).

It appears that Hdh1 and Hdh2 polypeptides have different common ancestors and evolved separately. The *Hdh1* gene is either retained in fission yeast and higher plants such as cabbage as a single gene or combined with the *hisI* and *hisE* genes encoding PRA-CH and PRA-PH to form a tri-functional histidine biosynthesis (*his*) gene in all other fungi. On the other hand, the *Hdh2* genes might be transferred and eventually became a non-functional residual DNA fragment in 3 *Aspergillus* and 2 *Phaeosphaeria* species. It appeared that the *Hdh2* genes were generally evolved from their common ancestor but lost in the majority of fungi during evolution.

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摘 要

王智立[1, 5]、林盈宏[2]、邱燕欣[3]、翁溥[4, 6]. 2008. 小麥葉枯病菌 *Phaeosphaeria nodorum* 組胺酸脫氫酵素僞基因之演化分析. 植病會刊 17：221-232 ([1] 鳳山熱帶園藝試驗分所 植物保護組；[2] 國立中興大學 植物病理學系；[3] 種苗改良繁殖場；[4] 美國農業部 (USDA) 分子植物病理研究室；[5] 現址：美國德州農工大學植物病理學暨微生物學系；[6] 聯絡作者，電子郵件：Peter.Ueng@ars.usda.gov；傳眞：+1-301-504-5449)

組胺酸脫氫酵素 (L-histidinol: NAD^+ dehydrogenase: HDH; EC 1.1.1.23) 是高等眞菌合成三功能-組胺酸 (tri-functional histidine, *his*) 的三種生合成酵素之一，於生合成組胺酸之最後兩個步驟中扮演催化的角色。本研究從小麥葉枯病菌 (Wheat-biotype *Phaeosphaeria nodorum*, PN-w) 與從波蘭裸麥分離之 *Phaeosphaeria* sp. (P-rye) 上鑑定出一單套之組胺酸脫氫酵素基因 (*Hdh2*)，但是此 *Hdh2* 基因並不存在於大麥葉枯病菌 (barley-biotype *P. nodorum*, PN-b) 菌株、燕麥殼針孢病菌 *P. avenaria* f. sp. *avenaria* (Paa)、麥類殼多胞斑點病菌 (*P. avenaria* f. sp. *triticea*, Pat1、Pat2、Pat3) 三群菌株，以及從達利雀稗 (dallis grass, *Paspalum* dilatatum Poir) 分離之 *Phaeosphaeria* sp. 菌株之中。由於 PN-w Sn37-1 的 *Hdh2* 基因於基礎培養基 (Minimal media) 與豐富培養基 (Rich medium) 上無法表現，得知 *Hdh2* 爲一僞基因。另外與受質結合以及2價鋅之結合配體 (Zn^{2+} ligand) 形成具重要性且與組胺酸脫氫酵素之活性有關之大部分胺基酸，完整保留於 *Hdh2* 蛋白中。藉由胺基酸序列推演其菌株間親緣性分析得知，在眞菌 HIS 蛋白的 *Hdh1* 胺基酸序列中，其組胺酸脫氫酵素之胺基酸位置，包括 C-116 及 C-153 皆爲半胱胺酸 (cysteine, C)，因而被分群至細菌群。而 3 個 *Aspergillus* 屬與 2 個 *Phaeosphaeria* 屬之 *Hdh2* 蛋白，在該組胺酸脫氫酵素的大部分分析中，C-116 的位置爲白胺酸 (leucine, L)，而 C-153 的位置全爲纈胺酸 (valine, V)，因此被分群至不同的細菌群。本文亦針對 *Aspergillus* 與 *Phaeosphaeria* 屬之 *Hdh2* 之基因演化進行討論。

關鍵詞：小麥葉枯病菌、小麥穎枯病、組胺酸脫氫酵素、僞基因、親緣分析