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The Tri-functional Histidine Biosynthesis Gene (*his*) in Wheat Stagonospora Nodorum Blotch Pathogen, *Phaeosphaeria nodorum*

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ABSTRACT

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The histidine biosynthesis gene (*his*) sequence was obtained from wheat-biotype *Phaeosphaeria nodorum* genomic DNA using a "step down" PCR amplification technique. The 2700-bp *his* gene fragment contained two exons and a 51-bp intron. The two exons of this gene encoded a complete 881-amino acid protein. Like the histidine synthesis proteins in other filamentous ascomycetes, the deduced protein contained the conserved domains for three biosynthetic activities: phosphoribosyl-AMP cyclohydrolase (PRA-CH; EC 3.5.4.19), phosphoribosyl-ATP pyrophosphohydrolase (PRA-PH; EC 3.6.1.31), and histidinol dehydrogenase (HDH; EC 1.1.1.23). The substrate and zinc ion binding location in this tri-functional histidine biosynthesis protein is discussed.

Key words: histidinol dehydrogenase, phosphoribosyl-AMP cyclohydrolase, phosphoribosyl-ATP pyrophosphohydrolase, *Phaeosphaeria nodorum*

Histidine is one of the essential amino acids which are not synthesized by mammals and obtained from their diet. In plants and microorganisms, histidine is mainly synthesized from two precursors, 5-phospho-D-ribosyl-1-pyrophosphate (PRPP) and ATP. Histidinol dehydrogenase (L-histidinol:NAD^+ oxidoreductase, EC 1.1.1.23) (HDH) is the enzyme that catalyzes the last two steps (from L-histidinol to histidine) in histidine biosynthesis. In bacteria, fission yeast (*Schizosaccharomyces pombe*), and higher plants, HDH is present as a single peptide, while the other two important enzymes, phosphoribosyl-AMP cyclohydrolase (PRA-CH; EC 3.5.4.19) and phosphoribosyl-ATP pyrophosphohydrolase (PRA-PH; EC 3.6.1.31), which catalyze the third and the second steps in the pathway, are combined in a single bi-functional polypeptide [1, 6, 13]. In other yeasts (*Saccharomyces cerevisiae* with *HIS4* gene) and many filamentous fungi (*Neurospora crassa* with *his-3* gene), PRA-CH, PRA-PH and HDH enzymes are aligned orderly as a tri-functional polypeptide which catalyzes histidine biosynthesis [4, 10].

Septoria disease of cereal is a disease complex caused by a number of fungi including two major pathogens

belonging to *Mycosphaerella* (anamorph; *Septoria*) and *Phaeosphaeria* (anamorph; *Stagonospora*) [16, 18]. Cereal *Septoria* pathogens are classified largely based on fungal morphology and host specificity [2, 3, 15]. Since the asexual stage pycnidiospore size of *Phaeosphaeria* species varies within single-spore cultures derived from the same isolate and is influenced by environmental conditions, their identification is complicated and difficult [8, 9]. Recently, molecular approaches have been used to study the phylogenetic relationships in *Phaeosphaeria* and other ascomycetes. The molecular tools may facilitate the identification of *Phaeosphaeria* pathogens in cereals [25]. It was reported that protein-coding genes may contribute to resolving the deep phylogenetic relationships in fungi [17]. Several genes encoding structural and functional important proteins such as β-glucosidase, glyceraldehyde-3-phosphate dehydrogenase, mating-type determinants and β-tubulin have been used for this purpose in cereal *Phaeosphaeria* species [11, 14, 23, 24]. Here, we reported the isolation of a tri-functional histidine biosynthesis gene (*his*) in wheat-biotype *Phaeosphaeria nodorum*. The potential presence of genetic diversities in nucleotide and its translated peptide sequences in the *his* gene may be useful for phylogenetic studies and species identification in the cereal pathogens causing Stagonospora leaf blotch diseases.

The wheat-biotype *P. nodorum* isolate Sn37-1 from Szelejewo, Poland was used for the *his* gene isolation. Procedures for fungal culture in a liquid medium and for genomic DNA (gDNA) isolation were described previously [22]. The *his* gene coding sequence was obtained with a "step down" PCR amplification technique [26]. A specific primer xy4B (CGAGAACGTTGTAAAAGAC GGACG) designed from a partial xylanase (endo-1, 4-β-xylanase; EC 3.2.1.8) (*xyl*) sequence (*unpublished data*) and primer pp1 (GTAATACGACTCACTATAGGGC) were used to amplify *NheI*-digested/adaptor 1-ligated genomic DNA mix. The primer pp1 recognized a sequence on adaptor 1 and served as the upstream primer for PCR [26]. A ca. 2.2 kb PCR fragment was eluted from agarose gel block after resolving by electrophoresis and sequenced (Fig. 1). Based on this known sequence, the primer 4x7 (CTATCGCGTTGTTAGCATTGCAC) was then designed and used along with pp1 for a second PCR. A ca. 2.5 kb fragment was amplified from the *Xba*I-digested/adaptor 1-ligated genomic DNA mix (Fig. 1). It appears that the *his* gene is ca. 1 kb upstream and closely located to one of the *xyl* genes in wheat-biotype *P. nodorum* (Fig. 1). By using the FGENESH program (http://www.softberry.com) with *Aspergillus* as the organism parameter, two exons and one 51-bp size intron (nt2547-nt2597 in Fig. 1) were predicted in the 2697-bp size *his* gene (accession no. DQ312266).

The 2646-bp nucleotide sequence in two exons of *his* gene from wheat-biotype *P. nodorum* encoded a protein of 881 amino acid residues. In higher plants, the histidine biosynthesis gene products are expressed as nuclear protein precursors and exported to the chloroplasts for biosynthesis of histidine [13]. Unlike the bi-functional PRA-PH and PRA-CH polypeptide in *Arabidopsis* and the HDH protein in cabbage (*Brassican oleracea* L.) [6, 13], which possess chloroplast transit peptide sequences in the N-terminals, the wheat-biotype *P. nodorum his* gene-encoded protein had no signal peptide as predicted with CBS Prediction Servers (http://www.cbs.dtu.dk).

Six tri-functional histidine biosynthesis peptide sequences of higher ascomycetes were retrieved from GenBank, including *P. nodorum* (DQ312266), *Magnaporthe grisea* (XP_363383), *Aspergillus nidulans* (XP_658401), *Neurospora crassa* (P07685), *Gibberella zeae* (XP_381041) and *Aspergillus fumigatus*

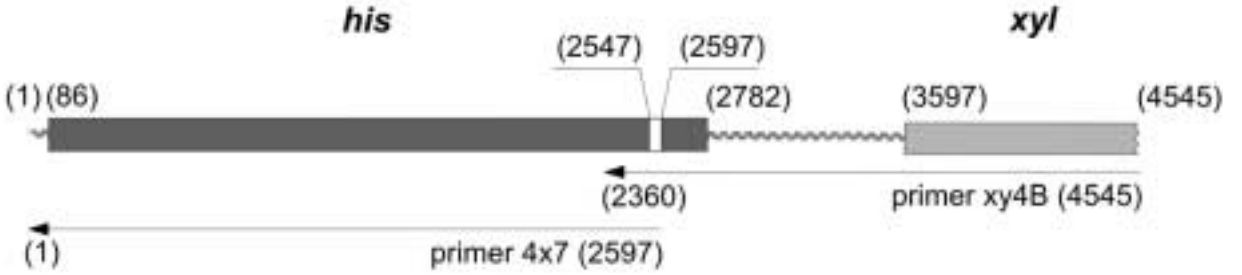
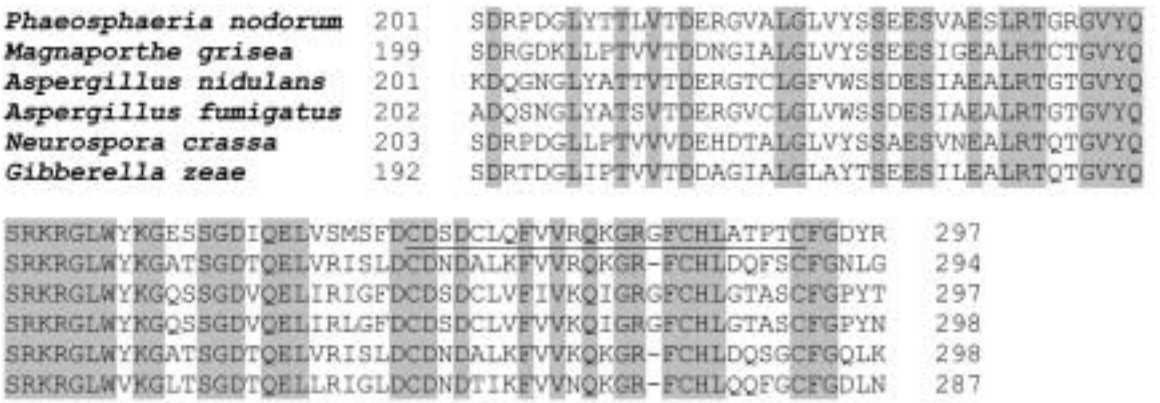
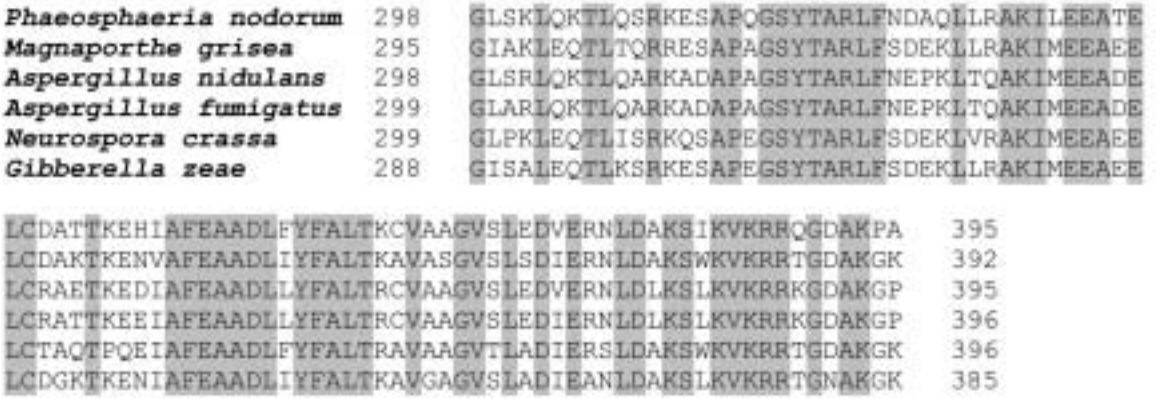


Fig. 1. Structure of tri-functional histidine biosynthesis gene (*his*; 'dark' box) in wheat-biotype *Phaeosphaeria nodorum*. The numbers in parentheses indicated to the nucleotide numbers (nt) of the sequenced PCR products.The 51-bp size intron in the *his* gene is positioned at nt2547 to nt2597. The partial sequence of xylanase gene (*xyl*) is shown in 'grey' box. The PCR products amplified by two oligonucleotide primers, 4x7 and xy4B, are indicated by arrows.

A. Phosphoribosyl-AMP cyclohydrolase (PRA-CH)



B. Phosphoribosyl-ATP pyrophosphohydrolase (PRA-PH)



C. Histidinol dehydrogenase (HDH)

(contn.)

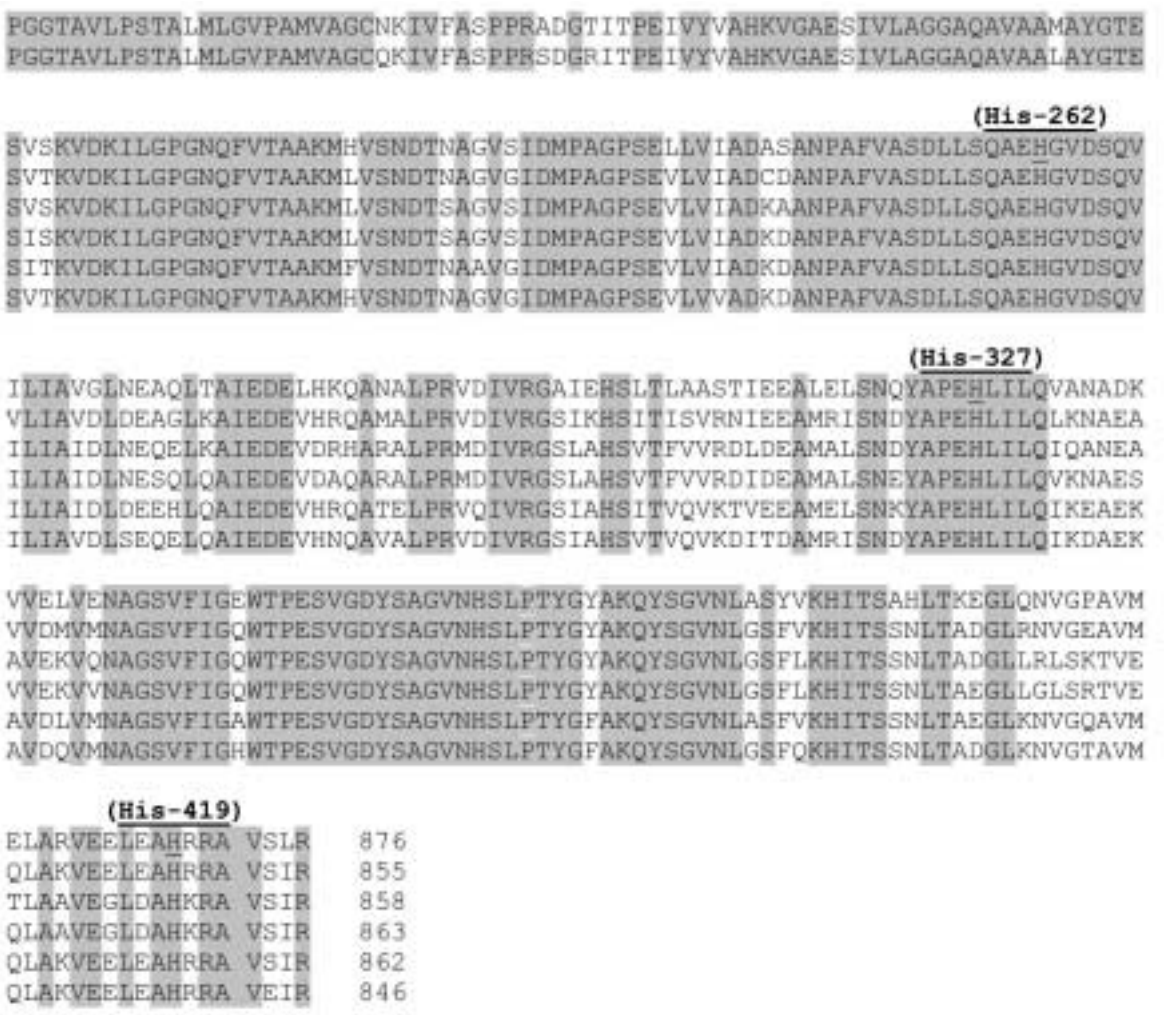


Fig. 2. Alignment of the deduced amino acid sequences of tri-functional histidine biosynthesis genes from representative filamentous ascomycetes. Six ascomycetes including wheat-biotype *Phaeosphaeria nodorum* (DQ312266), *Magnaporthe grisea* (XP_363383), *Aspergillus nidulans* (XP_658401), *Aspergillus fumigatus* (EAL90788), *Neurospora crassa* (P07685) and *Gibberella zeae* (XP_381041) were used for comparison. The amino acids conserved in the regions possessing enzymatic activities of phosphoribosyl-AMP cyclohydrolase (PRA-CH) (A), Phosphoribosyl-ATP pyrophosphohydrolase (PRA-PH) (B) and Histidinol dehydrogenase (HDH) (C), respectively, was shaded. The Zn binding motif in PRA-CH was underlined. In HDH, the substrate L-histidinol binding site (Cys-117) was boxed, and the zinc ion ligand formation sites (His-262, His-327 and His-419) were underlined. The GenBank accession numbers are given in parentheses.

(EAL90788). As compared to the counterpart in bacterium *Salmonella typhimurium*, the amino acid sites associated with substrate binding and catalytic activity were well conserved in the histidine biosynthesis tri-functional peptides in wheat-biotype *P. nodorum* and other ascomycetes (Fig. 2). Both the HDH and the PRA-CH peptides in bacteria have been reported to be zinc-activated metalloenzymes. The substrate L-histidinol binding site (Cys-117) and zinc ion ligand formation sites (His-262, His-327 and His-419), experimentally determined in the HDH of *Salmonella typhimurium* (accession no. P10370), were correspondingly found in the HDH of these ascomycetes (Fig. 2C) [7, 12, 19, 20]. Like in the PRA-CH in *Methanococcus vannielii*, a conserved region $C(X)_{15}CH(X)_5C$ for Zn ion binding and being responsible for catalytic activity, was also found in the putative PRA-CH conserved region of the histidine biosynthesis tri-functional polypeptides in these ascomycetes (Fig. 2A) [5].

Twelve histidine biosynthesis tri-functional peptides in ascomycetes, including that from wheat-biotype *P. nodorum*, were used for the study of phylogenetic relationships. The 795-881 amino acid sequences were aligned with ClustalX (1.83) in a multiple sequence alignment mode [21]. From the aligned sequences, 1,000 data sets were generated by bootstrap re-sampling in the 'seqboot' program of Phylogeny Inference Package

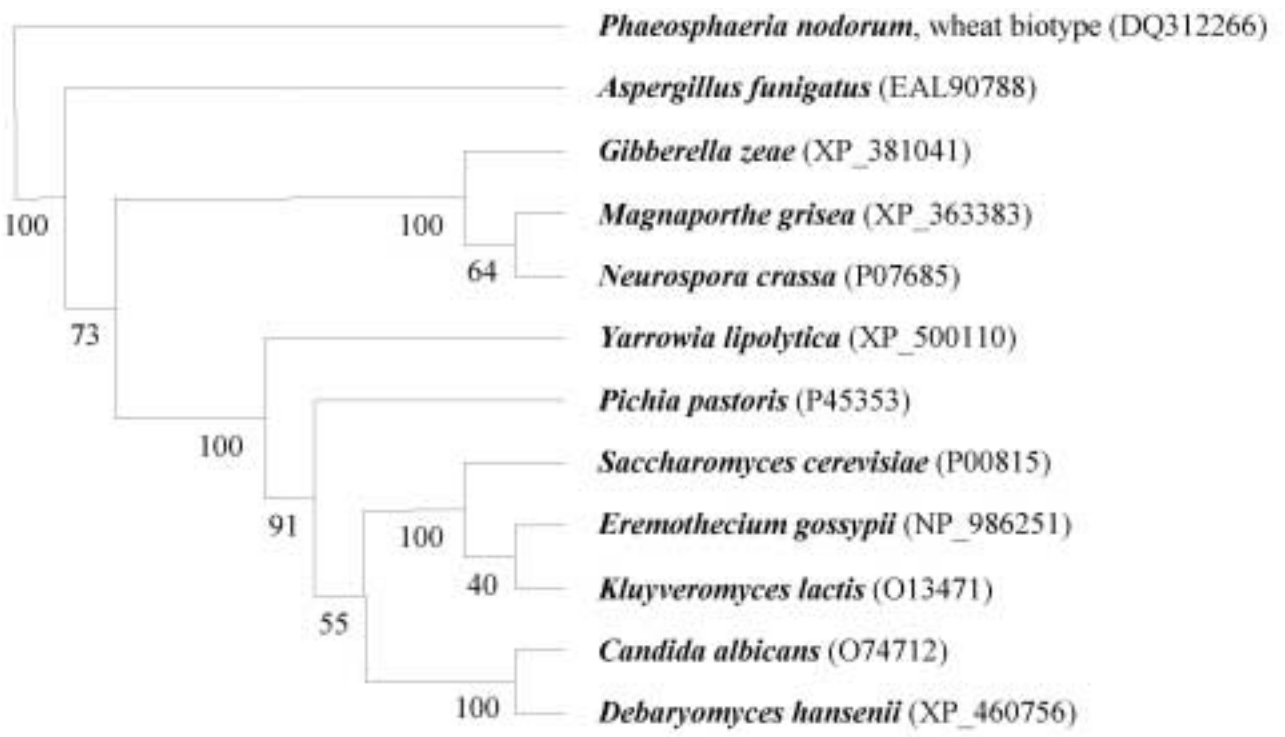


Fig. 3. Phylogenetic relationship based on the full-length amino acid sequences of tri-functional histidine biosynthesis polypeptides in ascomycetes. The GenBank accession numbers were given in parentheses. The Phylogeny Inference Package (PHYLIP) Version 3.6 (alpha2) was used for analysis. Bootstrap values (with 1000 replication) of the internal branches are indicated.

(PHYLIP) Version 3.6 (alpha2) (http://evolution.genetics.washington.edu/phylip.html). The bootstrapped data sets were evaluated by the maximum likelihood (ML) method using the 'proml' program for polypeptides. Finally, the 'consense' program was used to construct a 'tree'. It appeared that histidine biosynthesis peptides from yeasts and filamentous fungi formed two separate phylogenetic clades, and the *his* gene-encoded peptide in wheat-biotype *P. nodorum* was more closely related to that of *Aspergillus fumigatus* (Fig. 3). These data suggest that the tri-functional polypeptide sequences for histidine biosynthesis support the phylogenetic relations in ascomycetes.

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LITERATURE CITED

1. Apolinario, E., Nocero, M., Jin, M., and Hoffman, C.S. 1993. Cloning and manipulation of the *Schizosaccharomyces pombe his7*$^{+}$ gene as a new selectable marker for molecular genetic studies. Curr. Genet. 24:491-495.
2. Cunfer, B.M. 2000. *Stagonospora* and *Septoria* diseases of barley, oat, and rye. 2000. Can. J. Plant Pathol. 22:332-348.
3. Cunfer, B.M., and Ueng, P.P. 1999. Taxonomy and identification of *Septoria* and *Stagonospora* species on small-grain cereals. Annu. Rev. Phytopathol. 37:267-284.
4. Donahue, T.F., Farabaugh, P.J., and Fink, G.R. 1982. The nucleotide sequence of the *HIS4* region of yeast. Gene 18:47-59.
5. D'Ordine R.L., Klem T.J., and Davisson V.J. 1999. N1-(5'-phosphoribosyl)adenosine-5'-monophosphate cyclohydrolase: purification and characterization of a unique metalloenzyme. Biochemistry 38:1537-1546.
6. Fujimori, K., and Ohta, D. 1998. Isolation and characterization of histidine biosynthetic gene in Arabidopsis encoding a polypeptide with two separate domains for phosphoribosyl-ATP pyrophospho-hydrolase and phosphoribosyl-AMP cyclohydrolase. Plant Physiol. 118:275-283.
7. Grubmeyer C.T., and Gray W.R. 1986. A cysteine residue (cysteine-116) in the histidinol binding site of

histidinol dehydrogenase. Biochemistry 25:4778-4784.
8. Hooker, A.L. 1957. Cultural variability in *Septoria avenae* through successive single-macrospore transfers. Phytopathology 47:460-468.
9. Johnson, T. 1952. Cultural variability in *Septoria avenae* Frank. Can. J. Bot. 30:318-330.
10. Legerton, T.L., and Yanofsky, C. 1985. Cloning and characterization of the multifunctional *his-3* gene of *Neurospora crassa*. Gene 39:129-140.
11. Malkus, A., Reszka, E., Chang C.J., Arseniuk, E., Chang P.F.L., and Ueng, P. P. 2005. Sequence diversity of β-tubulin (*tubA*) gene in *Phaeosphaeria nodorum* and *P. avenaria*. FEMS Microbiology Lett. 249:49-56.
12. Nagai, A., and Ohta, D. 1994. Histidinol dehydrogenase loses its catalytic function through the mutation of $His^{261} \rightarrow$ Asn due to its inability to ligate the essential Zn. J. Biochem. 115:22-25.
13. Nagai, A., Ward, E., Beck, J., Tada, S., Chang, J.Y., and Scheidegger 1991. Structural and functional conservation of histidinol dehydrogenase between plants and microbes. Proc. Natl. Acad. Sci. USA 88:4133-4137.
14. Reszka, E., Chung, K.R., Tekauz, A, Malkus, A., Arseniuk, E., Krupinsky, J.M., Tsang, H., and Ueng, P.P. 2005. Presence of β-glucosidase (*bgl1*) gene in *Phaeosphaeria nodorum* and *Phaeosphaeria avenaria* f. sp. *triticea*. Can. J. Bot. 83:1001-1014.
15. Richardson, M.J., and Noble, M. 1970. Septoria species on cereals- a note to aid their identification. Plant Pathol. 19:159-163.
16. Scharen, A.L., and Sanderson, F.R. 1985. Identification, distribution and nomenclature of the Septoria species that attack cereals. p. 37-41 in "Septoria of cereals" by Scharen, A.L. (ed.), USDA-ARS, ARS-12, 116p.
17. Sicheritz-Ponten, T., and Andersson, S.G.E. 2001. A phylogenomic approach to microbial evolution. Nucleic Acids Res. 29:545-552.
18. Sprague, R. 1950. Diseases of cereals and grasses in North America. Ronald Press, New York.
19. Teng, H., and Grubmeyer, C. 1999. Mutagenesis of histidinol dehydrogenase reveals roles for conserved histidine residues. Biochemistry 38:733-7371.
20. Teng, H., Segura, E., and Grubmeyer, C. 1993. Conserved cysteine residues of histidinol dehydrogenase are not involved in catalysis. J. Biol. Chem. 268:14182-14188.
21. Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25:4876-4882.
22. Ueng, P.P., Bergstrom, G.C., Slay, R.M., Geiger, E.A., Shaner, G., and Scharen, A.L. 1992. Restriction fragment length polymorphisms in the wheat glume blotch fungus, *Phaeosphaeria nodorum*. Phytopathology, 82:1302-1305.
23. Ueng, P.P., Dai, Q., Cui, K., Czembor, P.C., Cunfer, B.M., Tsang, H., Arseniuk, E., and Bergstrom, G.C. 2003. Sequence diversity of mating-type genes in *Phaeosphaeria avenaria*. Curr. Genet. 43:121-130.
24. Ueng, P.P., Reszka, E., Chung, K.R., Arseniuk, E., and Krupinsky, J.M. 2003. Comparison of glyceraldehyde-3-phosphate dehydrogenase genes in *Phaeosphaeria nodorum* and *P. avenaria* species. Plant Pathology Bulletin 12:255-268.
25. Ueng, P.P., Subramaniam, K., Chen, W., Arseniuk, E., Wang, L., Cheung, A.M., Hoffmann, G.M., and Bergstrom 1998. Intraspecific genetic variation of *Stagonospora avenae* and its differentiation from *S. nodorum*. Mycol. Res. 102:607-614.
26. Zhang, Z., and Gurr, S.J. 2002. A "step down" PCR-based technique for walking into and the subsequent direct-sequence analysis of flanking genomic DNA. Methods in Molecular Biology 192:343-350. in "PCR Cloning Protocols, 2nd Edition", Chen, B.Y., and Janes, H.W., eds., Humana Press, Totowa, NJ.

摘　要

Malkus, A.[1]、鍾光仁[2]、張宗仁[3]、翁溥[4,5]. 2006. 小麥葉枯病菌 (*Phaeosphaeria nodorum*) 的三功能-組氨酸生合成基因. 植病會刊 15:55-62. ([1] 波蘭植物育種及馴化研究所植物病理學系；[2] 美國佛羅里達大學柑橘類作物研究及教育中心；[3] 美國喬治亞大學植物病理學系；[4] 美國農業部分子植物病理研究室；[5] 聯絡作者，電子郵件：uengp@ba.ars.usda.gov ；傳眞：+1-301-504-5449)

本研究利用「降溫式 (step down)」聚合酶連鎖反應 (PCR) 技術由小麥葉枯病菌 (*Phaeosphaeria nodorum*) 的基因組核酸中取得組氨酸生合成基因 (*his*) 序列。此 *his* 基因片段長度爲 2700 bp，包含二個外顯子 (exon) 及一個 51-bp 長的內含子 (intron)，這二個外顯子編碼 (encode) 了一個含 881 個氨基酸的完整蛋白質。如同其他絲狀子囊菌的組氨酸合成蛋白，小麥葉枯病菌 *his* 基因的推演 (deduced) 蛋白質中包含三個具生合成活性的保守性區域 (conserved domain)：磷酸核糖基-腺苷單磷酸環化水解酶 (phosphoribosyl-AMP cyclohydrolase ；PRA-CH ；EC 3.5.4.19)，磷酸核糖基-腺苷三磷酸焦磷酸水解酶 (phosphoribosyl-ATP pyrophosphohydrolase ；PRA-PH ；EC 3.6.1.3.1)，與組氨酸脫氫酶 (histidinol dehydrogenase ；HDH ；EC 1.1.1.23)。文中也將討論此三功能-組氨酸生合成蛋白與其受質和鋅離子的結合位置。

關鍵詞：小麥葉枯病菌、組氨酸脫氫酶、磷酸核糖基-腺苷三磷酸焦磷酸水解酶、磷酸核糖基-腺啓單磷酸環化水解酶