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sid1, a gene initiating siderophore biosynthesis in *Ustilago maydis*: Molecular characterization, regulation by iron, and role in phytopathogenicity

(L-ornithine N^5-oxygenase/ferrichrome/nucleotide sequence/intron/GATA)

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ABSTRACT **Iron uptake in *Ustilago maydis* is mediated by production of extracellular hydroxamate siderophores. L-Ornithine N^5-oxygenase catalyzes hydroxylation of L-ornithine, which is the first committed step of ferrichrome and ferrichrome A biosynthesis in *U. maydis*. We have characterized *sid1*, a gene coding for this enzyme, by complementation in trans, gene disruption, and DNA sequence analysis. A comparison of genomic DNA and cDNA sequences has shown that the gene is interrupted by three introns. The putative amino acid sequence revealed similarity with *Escherichia coli* lysine N^6-hydroxylase, which catalyzes the hydroxylation of lysine, the first step in biosynthesis of aerobactin. Two transcription initiation points have been determined, both by PCR amplification of the 5′ end of the mRNA and by primer extension. A 2.3-kb transcript which accumulates in cells grown under low iron conditions was detected by Northern hybridization. A less abundant 2.7-kb transcript was observed in cells grown in iron-containing medium. By contrast, constitutive accumulation of the 2.3-kb transcript was observed in a mutant carrying a disruption of *urbs1*, a gene involved in regulation of siderophore biosynthesis. Analysis of the pathogenicity of mutants carrying a null allele of *sid1* suggests that the biosynthetic pathway of siderophores does not play an essential role in the infection of maize by *U. maydis*.**

Siderophores are low molecular weight compounds produced by microorganisms to scavenge iron from the environment under conditions of iron stress (1). Siderophores have also been implicated as virulence factors for some animal and human pathogens (2). However, there is limited knowledge regarding the role that siderophores play in the development of plant disease (3). We are conducting a systematic analysis of the siderophore-mediated iron uptake system of *Ustilago maydis* in order to assess its role in pathogenicity.

U. maydis, the causative agent of corn smut disease, produces the siderophores ferrichrome and ferrichrome A (4). The siderophores are cyclic hexapeptides each composed of three residues of δ-*N*-acyl-δ-*N*-hydroxyornithine and either three residues of glycine (ferrichrome) or two residues of serine and one of glycine (ferrichrome A) (5).

Based on the information from work on biosynthesis of ferrichrome in *Ustilago sphaerogena* (5) and rhodotorulic acid in *Rhodotorula pilimanae* (6), ferrichrome biosynthesis is suggested to proceed by hydroxylation of ornithine and then acetylation to give δ-*N*-acetyl-δ-*N*-hydroxyornithine. The latter steps in ferrichrome synthesis are unknown but may proceed via covalently bound thioester intermediates on a multifunctional polypeptide.

To begin an analysis of siderophore biosynthesis in *U. maydis*, mutants defective in biosynthesis of siderophores were isolated and characterized (7). Class II mutants are blocked in the biosynthesis of δ-*N*-hydroxyornithine. A cosmid clone capable of complementing the class II mutants was identified from a genomic DNA library of a wild-type strain of *U. maydis* (7). These results confirmed that L-ornithine and *N*-hydroxyornithine are common precursors of ferrichrome and ferrichrome A and that the first step of the siderophore biosynthesis is the hydroxylation of L-ornithine catalyzed by L-ornithine N^5-oxygenase.

In this report, we describe the localization and characterization of *sid1*, a gene encoding L-ornithine N^5-oxygenase on cloned genomic DNA. The isolation and cloning of a full-length cDNA for this enzyme are also presented, and some features of the DNA sequence are discussed.§ We also describe our initial analysis of *sid1* regulation and its role in phytopathogenicity.

MATERIALS AND METHODS

Strains and Media. *Escherichia coli* DH5α (Bethesda Research Laboratories) was used for all DNA manipulations. The *Salmonella typhimurium* LT-2 mutant, *enb-7*, was a gift from J. B. Neilands (University of California, Berkeley). The *U. maydis* strains employed are listed in Table 1. Media for the culture of *U. maydis* have been described (8). Low-iron (LI) medium and ME medium for the bioassay of siderophore production have also been described (7).

DNA Manipulations. Plasmid DNA was prepared from *E. coli* by the boiling minipreparation protocol (9) or by the differential ammonium acetate precipitation method (10). *U. maydis* genomic DNA was isolated by the glass-bead method (11). Vectors for subcloning were pHL1, an integrative vector (12), and pCM54, an autonomously replicating vector (13). DNA transformation of *U. maydis* was carried out as described by Wang *et al.* (12) and modified by C. Voisard (C. Voisard and S.A.L., unpublished work). Transformation of *E. coli*, Southern hybridization, and other DNA manipulations were performed as described (14). Nucleotide sequencing was by the dideoxy method using Sequenase (United States Biochemical) and synthetic oligonucleotide primers based on previously determined sequences.

RNA Isolation and Northern Blot Analysis. *U. maydis* total RNA was extracted by the method of Kohrer and Domdey (15). Residual DNA was removed by CsCl centrifugation (16). For Northern analysis, RNA samples were glyoxylated and fractionated by electrophoresis in a 1.2% agarose gel

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§The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M98520 (genomic DNA) and M98523 (cDNA)].

Table 1. Strains of *U. maydis*

Strain	Relevant characteristics
518*	Wild type *a2b2*
521*	Wild type *a1b1*
S023†	*sid1 a1b1*
S018‡	*sid1 pan1-1 ino1-3 nar1-1 rec1-1 a1b1*
SH^{r}008§	*sid1::hyg^r a2b2*
SH^{r}004§	*sid1::hyg^r*
SH^{r}031§	*sid1::hyg^r ade1-1 met1-2 nar1-1 rec2-1 a2b2*
C013¶	*urbs1::hyg^r a2b2*

U. maydis strains obtained from R. Holliday (Commonwealth Scientific and Industrial Research Organization Laboratory for Molecular Biology, Sydney, Australia).
†Obtained from wild type by mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, as described (7).
‡Constructed from cross a between S023 and 277 (*pan1-1 ino1-3 nar1-1 rec1-1 a1b1*).
§Constructed by disruption of *sid1* as described in the text.
¶Constitutive mutant constructed by gene disruption (C. Voisard and S.A.L., unpublished work).

containing 10 mM sodium phosphate buffer (pH 7.0) (14). RNA was transferred to Nytran membranes (Schleicher & Schuell) and then hybridized and washed under stringent conditions (14).

Screening of cDNA Library. A cDNA library from *U. maydis* grown in LI medium was constructed in the phage vector λZAPII by C. Voisard (C. Voisard and S.A.L., unpublished work). The cDNA library was screened by hybridization with a 3.2-kb *Ssp* I–*Eco*RI genomic DNA fragment (Fig. 1). The positive clones were subcloned into the pBluescript vector by *in vivo* excision from the recombinant λZAPII phage using the protocol provided by the manufacturer (Stratagene).

Primer Extension Mapping and Cloning the 5' End of *sid1* mRNA. Primer extension with primers 1 and 2 (Fig. 2) was performed as described (17). 5'-RACE (rapid amplification of cDNA ends) was used to clone the 5' end of *sid1* cDNA (18). Total RNA from *U. maydis* cells grown in LI medium was reverse-transcribed with primer 3 (Fig. 2). The cDNA products were isolated and tailed with poly(A) by using dATP and terminal deoxynucleotidyltransferase. Second-strand synthesis was carried out with a $(dT)_{17}$ primer–adaptor (18). The double-stranded cDNA product was amplified by PCR (polymerase chain reaction) using the adaptor of the $(dT)_{17}$ primer–adaptor and internal primer 4 (Fig. 2) attached to a 10-nucleotide *Eco*RI adaptor. PCR conditions were 30 cycles at 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, followed by 72°C for 5 min. The PCR products were subcloned in a pBluescript vector. Phagemids with insert were identified by colony hybridization (14) and then sequenced.

Isolation of Fungal Siderophores. Ferrichrome and ferrichrome A were detected by the ferric perchlorate assay (4).

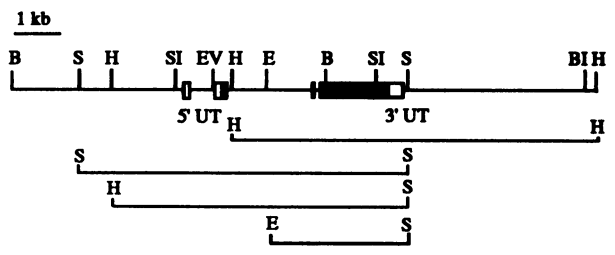

FIG. 1. Structure of *sid1* and restriction map. Only sites used for subcloning are indicated (B, *Bgl* II; BI, *Bgl* I; E, *Eco*RI; EV, *Eco*RV; H, *Hin*dIII; S, *Ssp* I; SI, *Sst* I). Exons are boxed. Coding regions are indicated as black boxes, whereas 5' and 3' untranslated regions (UT) found in the cDNA clone are indicated by open boxes. Subcloned fragments mentioned in the text are shown below the map.

Siderophores were extracted from culture supernatants and analyzed by thin-layer chromatography (TLC) or high-pressure liquid chromatography (HPLC) as described (4).

Genetic Manipulations. Genetic crosses were performed by standard methods (7, 19). Fungal cell suspensions (10^6 cells per ml) of compatible mating type were injected (≈0.1 ml per plant) into seedlings of the maize cultivar Golden Cross Bantam (20).

Enzyme Assay. *U. maydis* cells grown in LI medium with or without 10 μM $FeSO_4$ were harvested, disrupted by passage through a French pressure cell at 18,000 psi, and then centrifuged at 12,000 × *g* for 30 min. Enzyme activity of the supernatant was measured according to Plattner *et al.* (21), except that lysine was replaced by L-ornithine as a substrate and the reaction temperature was set at 28°C for 2 hr. Under these conditions the enzyme activity was linear with time. Products of the reaction were determined using a modified Csaky test (22). Protein was determined by the biuret method (23).

RESULTS

Location of *sid1* on Cloned DNA. From previously reported experiments, pSid1, a cosmid clone from a genomic library of wild-type *U. maydis*, was believed to contain the *sid1* gene (7). The restriction map of a 12.5-kb region of pSid1 containing an 8.1-kb *Hin*dIII fragment which restores siderophore production in the *sid1*$^-$ strain S023 is presented in Fig. 1. Initial subcloning of the region indicated that a 2.5-kb *Hin*dIII–*Nru* I fragment contained the complementing activity when bioassay methods were used to confirm the presence of siderophores (7). Inability to confirm siderophore production in culture supernatants by chemical analysis indicated a need to reexamine the 8.1-kb fragment. The smallest DNA fragment conferring complementation via integrative transformation was contained within a 3.2-kb *Ssp* I–*Eco*RI (Fig. 1). However, neither the 3.2-kb *Ssp* I–*Eco*RI nor the 8.1-kb *Hin*dIII fragments complemented S023 in trans. A 7.2-kb *Ssp* I fragment overlapping the 3.2-kb *Ssp* I–*Eco*RI region (Fig. 1) was subcloned into pCM54 and designated pBM100; this construct complemented S023 in trans (Table 2). TLC and HPLC analysis confirmed that biosynthesis of ferrichrome and ferrichrome A had been restored in the transformants harboring pBM100 (data not shown). Further subcloning experiments (Fig. 1) indicated that a 6.5-kb *Ssp* I–*Hin*dIII fragment of pBM100 was the smallest DNA fragment that could complement S023 in trans. Little or no activity of L-ornithine N^5-oxygenase was found in extracts of the wild-type cells grown in LI medium amended with iron or in that of S023 cells grown in LI medium with or without iron (Table 2). Enzyme activity under LI growth condition was restored in S023 transformed with pBM100, while disruption of *sid1* by insertion of the hygromycin B-resistance gene (see below) abolished enzyme activity. Little activity was detected in the extract of the transformant bearing pBM100 when cultured under iron-replete conditions. This indicated that both L-ornithine N^5-oxygenase activity and siderophore production in the transformant strain were under the same regulatory constraints as in the wild type.

Disruption of *sid1*. A 3-kb blunt-ended *Hin*dIII fragment carrying the selectable marker for resistance to hygromycin B (12) was inserted into the single *Bgl* II site of the 7.2-kb *Ssp* I fragment encompassing *sid1* (Fig. 1) and used to transform the haploid wild-type strain. The replacement of the genomic 7.2-kb *Ssp* I fragment with the 10.2-kb fragment by homologous recombination was verified by Southern hybridization analysis (data not shown). The transformants in which gene replacement had taken place also lacked L-ornithine N^5-oxygenase activity and siderophore production (Table 2). Crosses between the disruption strains and wild-type strain

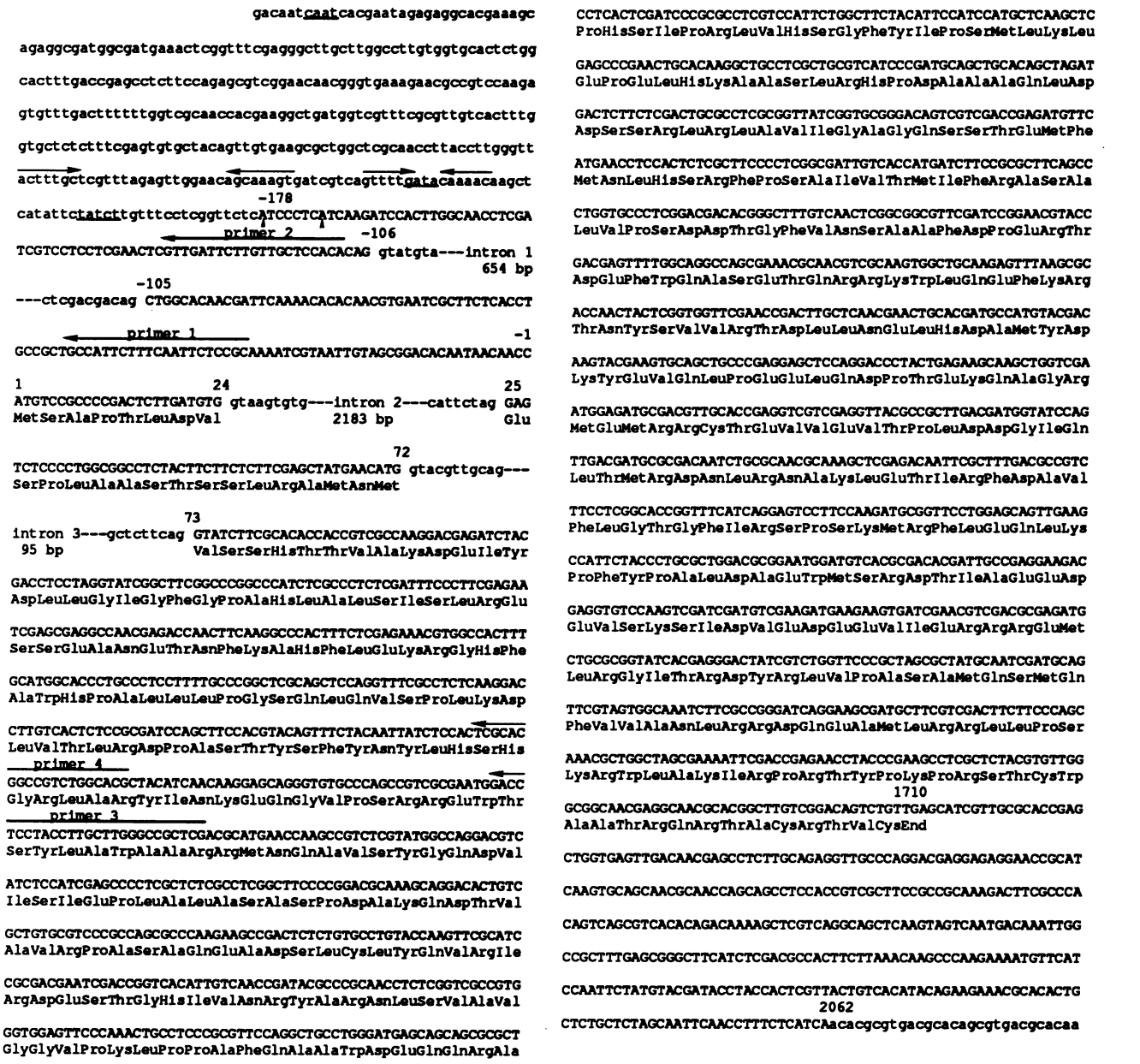

FIG. 2. Nucleotide sequence of *sid1* and deduced amino acid sequence. Nucleotide residues are numbered on the basis of the cDNA sequence from +1 at the adenine of the translational initiator codon, with nucleotides in the 5′ untranslated region indicated by negative numbers. The putative CAAT box and GATA sequences are underlined. Lines are positioned above the inverted repeat sequences. The cDNA sequence is presented in capital letters, whereas the 5′ upstream region of the gene, partial intron sequences, and 3′ genomic region are displayed in lowercase letters. Arrows indicate the transcription start sites. The poly(A) stretch starts from the A at 2062. Primers (complementary to the cDNA nucleotide sequence) used for 5′ RACE and primer extension are indicated as arrows with the respective primer number.

gave a 1:1 ratio of *sid1*$^+$ and *sid1*$^-$ haploid basidiospore segregants (Table 3) as would be expected for the segregation of alternative alleles of a single locus. Hygromycin B resistance cosegregated with the defect in siderophore biosynthesis. Southern hybridization of genomic DNA from selected segregants confirmed that the mutant phenotype cosegregated with the 10.2-kb fragment (data not shown). A diploid strain constructed with S018, an *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine-induced *sid1* mutant, and SH^{r}031, a strain carrying a disruption in *sid1*, was unable to produce siderophores and yielded only *sid1*$^-$ progeny (Table 3), indicating that the locus mutated by the alkylating agent was the same as that disrupted by the hygromycin B-resistance gene.

Cloning of the cDNA and Determination of the mRNA 5′ Ends. The *U. maydis* cDNA library was initially screened with the 3.2-kb *Ssp* I–*Eco*RI genomic DNA fragment (Fig. 1). DNA sequencing of eight positive clones indicated that these clones had consistent 3′ ends with poly(A) tails and sequences corresponding to part of the 7.2-kb *Ssp* I genomic fragment. The largest cDNA clone was 1.8 kb. To clone the 5′ end of *sid1* mRNA, the cDNAs resulting from primer extension with primer 3 were amplified by PCR and cloned into a pBluescript vector. Among the population of the cDNA clones, there were two sizes differing by 6 nucleotides in length at the 5′ end, suggesting that the 5′ ends of the *sid1* mRNA were heterogeneous. The two transcription initiation sites (Fig. 2) of the gene were defined by primer extension analysis with primers 1 and 2 (data not shown).

Nucleotide Sequence and Structure of *sid1*. The nucleotide sequence of a 2240-bp *sid1* cDNA and the deduced amino acid sequence are shown in Fig. 2. The cDNA sequences are identical to parts of the 6.5-kb *Ssp* I–*Hin*dIII genomic frag-

Table 2. Effect of iron on siderophore production and activity of L-ornithine N^5-oxygenase in *U. maydis*

Strain	Siderophore production, OD_{495}		Enzyme activity, nmol/min per mg of protein	
	− Fe	+ Fe	− Fe	+ Fe
518	0.28	0.03	0.21	0.04
S023	0.03	0.04	0.01	ND
SH^{r}008	0.03	0.03	0.00	ND
S023/pBM100	0.25	0.01	0.16	0.02

Cells were grown in LI medium with or without 10 μM $FeSO_4$ at 28°C for 3 days and collected by centrifugation. Siderophore production in the supernatant was measured by the ferric perchlorate assay. The enzyme assay is described in *Materials and Methods*. Strain S023/pBM100 is the mutant S023 transformed with plasmid pBM100. ND, not determined.

ment and show that the gene is interrupted by three introns of 654, 2183, and 95 bp, respectively (Fig. 2). All intron/exon borders show good agreement with the consensus splice-site sequences [5′-GGTAaGT...(C/T)AG-3′)] for introns of filamentous fungi (24), although a "lariat formation" consensus sequence was found only in intron 2.

The mRNA has a 178-nucleotide 5′ untranslated region, an open reading frame coding for a protein of 570 amino acids, and a 352-nucleotide 3′ untranslated region preceding the poly(A) tail. The putative translation initiation site (CAACCATGTC) matches the consensus sequence [CCA(C/A)(C/A)ATGGC] for a fungal translation initiation site; the −3 position is always a purine and is usually an adenine (24). The 5′ nontranscribed region of the gene does not contain a TATA box, and a CAAT box is present 350 bp farther upstream than usual. There are, however, pyrimidine-rich regions between positions −186 and −197 and between positions −315 and −323, which could conceivably contribute to the initiation of transcription, as is the case for other genes of filamentous fungi (24). Sequences recognized by the GATA family of zinc finger transcription factors, TATC and TGATAC, are located at −196 and −218 (Fig. 2). Interestingly, two inverted repeat sequences are found in the region immediately upstream of the transcriptional start sites. Of the two dyad symmetry sequences, one contains a GATA sequence in its loop region.

A FASTA (25) comparison of the deduced amino acid sequence with the Genpept data bank (release no. 71.0) revealed similarity to a functional analogue, lysine N^6-hydroxylase encoded by the *E. coli iucD* gene (26). The two proteins are 35% identical over a 124-amino acid overlap at the N terminus; when conservative amino acid substitutions are included, the sequence similarity is >74% (Fig. 3).

Regulation of *sid1* by Iron. To determine whether iron affects the accumulation of *sid1* mRNA, a Northern blot analysis of total RNA isolated from wild type and a constitutive mutant (C013) grown in LI medium with or without 10 μM $FeSO_4$ was performed (Fig. 4). A high level of the 2.3-kb transcript was observed in wild-type cells grown in LI medium, while a less abundant transcript of 2.7-kb was found

Table 3. Segregation of the hygromycin B-resistance gene linked to *sid1*

Cross	*sid1*$^-$/*sid1*$^+$ segregants*	hyg^r/hyg^s segregants
518 × 521	0/40	0/40
518 × S023	26/24†	0/50
SH^{r}008 × 521	105/95†	105/95†
SH^{r}031 × S018	324/0	164/160†

*Determined by bioassay with *S. typhimurium enb-7*.
†A χ^2 analysis for a 1:1 model (one locus, two alleles) yielded $P > 0.4$.

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          10        20        30        40        50        60
sid1  VESPLAASTSSLRAMNMVSSHTTVAKDEIYDLLGIGFGPAHLALSISLRESSEANETNFK
                                    :..:.: :: .:... .:..  :    ...
iucD                              MKKSVDFIGVGTGPFNLSIA-ALSHQIE----ELD
                                       10        20              30

          70        80        90       100       110       120
sid1  AHFLEKRGHFAWHPALLLPGSQLQVSPLKDLVTLRDPASTYSFYNYLHSHGRLARYINKE
       :.... ::.:::..:.:....:.  :::::.  .:...::: ::: .: .. :....
iucD  CLFFDEHPHFSWHPGMLVPDCHMQTVFLKDLVSAVAPTNPYSFVNYLVKHKKFYRFLTSR
                40        50        60        70        80        90

          130       140       150       160       170       180
sid1  QGVPSRREWTSYLAWAARRMNQAVSYGQDVISIEPLALASASPDAKQDTVAVRPASAQEA
        . :: :...:: ::: ::  . ....: .:.
iucD  LRTVSREEFSDYLRWAAEDMNN-LYFSHTVENIDFDKKRRLFLVQTSQGQYFARNICLGT
                100       110       120       130       140
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FIG. 3. Sequence comparison of L-ornithine N^5-oxygenase (*sid1*) and lysine N^6-hydroxylase (*iucD*). Two dots between aligned amino acids indicate an exact match in the sequence; one dot denotes a conservative replacement. Dashes within the coding sequences indicate spaces inserted into one sequence by the FASTA program to achieve optimal alignment of the two proteins. The putative FAD-binding sequence is underlined.

in the cells grown in the presence of iron. By contrast, approximately the same levels of the 2.3-kb transcript were observed in C013 cells grown in low or high iron conditions. No signals were detected in total RNA isolated from the *U. maydis sid1* disruption mutant grown in the presence or absence of iron (data not shown).

Pathogenicity Test. Experiments were conducted to assess the phytopathogenicity of siderophore-nonproducing class II mutants. A mixture of haploid, *sid1*$^-$ progenies of compatible mating type were used to inoculate maize seedlings and resulted in disease development without significant difference in disease ratings relative to those obtained from infection with wild-type strains (28). These experiments were repeated with compatible strains disrupted in *sid1*. The disrupted strains from 518 and 521 displayed plate mating activity, and their growth was severely impaired in LI medium (data not shown). To determine pathogenicity, mutant cells were cultured in LI medium or LI medium amended with 10 μM $FeSO_4$, and compatible mixtures were injected into maize seedlings at concentrations of 10^5 or 10^6 cells per ml. Disease ratings of plants 14 days after inoculation indicated that the disruption of *sid1* did not significantly alter the phytopathogenicity of *U. maydis* under these experimental conditions (Table 4).

DISCUSSION

In this report, we characterize the *U. maydis* gene for L-ornithine N^5-oxygenase at the molecular level. The proof of the identity of the gene includes (*i*) restoration of L-ornithine N^5-oxygenase activity and the ability to produce siderophores in *sid1* mutants by trans-complementation, (*ii*) abolishment of siderophore production and L-ornithine N^5-

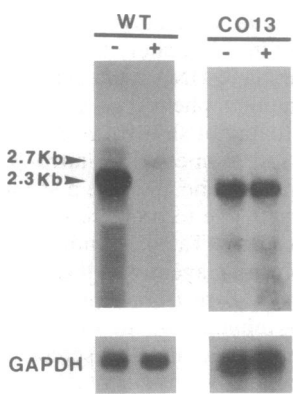

FIG. 4. Northern blot analysis of *sid1* mRNA. Total RNA was isolated from a wild-type strain (WT) or constitutive mutant (C013) grown in LI medium without (−) or with (+) 10 μM $FeSO_4$. Ten micrograms of total RNA from each sample was prepared as described in *Materials and Methods* and probed with ^{32}P-labeled 7.2-kb *Ssp* I genomic DNA (*Upper*). The same blots were stripped and reprobed with DNA encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from *U. maydis* (27), as an internal standard (*Lower*).

Table 4. Pathogenicity of wild-type and disrupted mutant strains of *U. maydis*

Strain	$FeSO_4$ added*	Inoculum, cells per ml	Disease rating at 14 days
521 × 518	−	10^5	3.5
	−	10^6	4.5
	+	10^5	3.5
	+	10^6	3.9
$SH^r004 \times SH^r008$	−	10^5	3.2
	−	10^6	3.4
	+	10^5	3.3
	+	10^6	4.2

Cells from each cross were injected into 20–24 maize seedlings. Disease symptoms were scored as described (20).
*Cells were grown in LI medium with (+) or without (−) 10 μM $FeSO_4$.

oxygenase activity by gene disruption, and (*iii*) allelism of chemically and molecularly created *sid1* mutations. In addition, the deduced amino acid sequence of *sid1* cDNA shows significant similarity to L-lysine N^6-hydroxylase (Fig. 3), an enzyme which carries out an analogous reaction in aerobactin siderophore biosynthesis in *E. coli* (21). Such structural similarities most likely provide the basis for the functional equivalence of the proteins and probably represent an important functional domain for a putative FAD-binding sequence. L-Ornithine N^5-oxygenase does not contain the putative NADPH-binding sequence that is found in lysine N^6-hydroxylase; however, the hydropathy profiles show that four regions of apparent hydrophobicity are conserved at the N terminus (data not shown).

The *sid1* gene is split by three introns (Fig. 2). The 2183- and 654-bp introns are much larger than the 48- to 398-bp introns reported for other filamentous fungal genes (24). A second cDNA species was found which is located in intron 2 and overlaps exon 2 of *sid1* (unpublished observation). Although the function of corresponding mRNA is currently unknown, this finding could account for the unusually large 2.2-kb intron in the gene.

High levels of a 2.3-kb transcript were detected in wild-type cells grown in LI medium, whereas a less abundant 2.7-kb transcript was found in the cells grown in LI medium with iron (Fig. 4), suggesting that the levels of *sid1* mRNA may be affected both transcriptionally and posttranscriptionally. For example, the ferric reductase gene of yeast is transcriptionally regulated by iron (29), while the stability of human transferrin receptor mRNA is regulated by the intracellular level of iron (30). No sequence motifs similar to the iron regulatory element of the human transferrin receptor mRNA (30) were observed in the transcribed DNA of *sid1*. The presence of a larger transcript in cells grown under high iron conditions may indicate that iron-mediated regulation of *sid1* is also at the level of mRNA splicing. Another possibility is that two iron-regulated promoters differentially control expression of the gene. A final interpretation is that these messages represent overlapping genes. Further analysis of the 2.7-kb transcript should distinguish between these or other possibilities.

In contrast to the wild type, the same high levels of the 2.3-kb transcript were observed in the total RNA isolated from cells of the *urbs1* mutant C013 whether grown in high-iron or LI medium. The deduced amino acid sequence from *urbs1* contains two putative zinc finger motifs (C. Voisard and S.A.L., unpublished work) that have been associated with binding of DNA in transcription factors of the GATA family (31–33). The promoter region of *sid1* has two GATA boxes, the recognition sequence for this class of transcription regulatory proteins, suggesting that the *sid1* gene may be one of the *urbs1* target genes in the biosynthetic pathway of siderophores in *U. maydis*.

Results from the use of *sid1* null mutants revealed no correlation between phytopathogenicity and the biosynthetic system for ferrichrome siderophores in *U. maydis*. However, this conclusion does not take into account the possible role in pathogenicity of another iron transport system in *U. maydis*, such as that found in *Saccharomyces cerevisiae*, which utilizes a ferric reductase activity to transport iron into the cells (29). Another consideration is that the conditions used here to assess phytopathogenicity do not mimic those found in the field.

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