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Genetic diversity and transcriptional analysis of the *bys1* gene from *Blastomyces dermatitidis*

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Abstract

Blastomyces dermatitidis, a pathogenic fungal organism, is able to exist in two different morphologies, a multicellular mycelium or a unicellular yeast, according to temperature, 25 °C and 37 °C respectively. The switching between morphologies must be accompanied by a cascade of signaling events in which expression of genes responsible for the change of morphology is increased or decreased. *bys1*, a gene from *B. dermatitidis* isolate #58, is expressed at high levels in the unicellular yeast, but gradually diminishes as the temperature is lowered and the organism converts to the mycelial phase where there is no transcription of *bys1*. We explored if *bys1* homologs are found in other *B. dermatitidis* isolates and if the transcription of the homologs were regulated by temperature. *bys1* was identified in all *B. dermatitidis* isolates tested and could be grouped into two classes by Southern blot, PCR, and DNA sequence. Although the *bys1* transcripts of both classes were regulated by temperature, transcription rates varied between the three isolates tested.

Key words:*Blastomyces dermatitidis*, *bys1*, dimorphism, phase-specific gene expression

Introduction

The dimorphic fungus *Blastomyces dermatitidis*, the etiological agent of the systemic fungal disease blastomycosis, grows as a saprophytic multicellular mycelium in the environment. When the mold is disturbed, aleuriocondia are released into the air where they are inhaled into the lung of a susceptible mammalian host. Once inside the host, the conidia undergo a morphological change to the pathogenic unicellular yeast. Healthy individuals as well as those who are immunocompromised can become infected. The primary infection displays considerable variability from an asymptomatic infection to an acute self-limiting pulmonary infection which can progress to extrapulmonary dissemination and possibly chronic disease [1].

In North America, *B. dermatitidis* is endemic to the eastern United States, the Mississippi and Ohio River valleys and extending northward to the Great Lakes and southern Canada. *B. dermatitidis* is also endemic in Africa and has been reported in India [2]. Dogs are the most common mammal infected, but humans and other mammals are also susceptible [3–5].

A phenotype of *B. dermatitidis* and other dimorphic fungi are their ability to change morphology from the saprophytic mycelium phase to the pathogenic yeast phase. The change in morphology associated with inhalation of the spore into the lung can be mimicked in the laboratory by growing the organism at 25 °C and then raising the culture temperature to 37 °C. Currently, very little is known about the genetic or biochemical mechanisms responsible for the morphological change in *B. dermatitidis*. During the first 24 hours at 37 °C, *B. dermatitidis* undergoes first, a heat shock response, uncoupling of oxidative phosphorylation and a decline in ATP and respiration rates, and secondly, spontaneous respiration then ceases fol-

lowed by a recovery period and the transition of the mycelial phase to the yeast phase [6, 7].

The *B. dermatitidis* nuclear genome is estimated to be composed of four chromosomes and to be 28 Mb in size [8]. Presumably, many genes are important for mycelial or yeast phase growth, the transition from one phase to the other, or pathogenic factors for causing an infection in mammals. *bys1*, a gene identified from *B. dermatitidis* isolate #58 whose function is unknown, has been shown to be transcribed in the unicellular yeast phase, but not the mycelial [9]. The mRNA level of *bys1* increases with time when the culture temperature is raised from 25 °C to 37 °C and decreases when the temperature is lowered to 25 °C from 37 °C.

In this study, we surveyed other *B. dermatitidis* isolates for *bys1* homologs. PCR, Southern blot and nucleotide sequence analysis identify two *bys1* alleles among all the *B. dermatitidis* isolates surveyed and that each *B. dermatitidis* isolates possessed only one of them. Northern blot analysis of the two *bys1* alleles revealed that both are only transcribed at 37 °C but the kinetics of transcriptional activation were different.

Materials and methods

Fungal strains and media

B. dermatitidis isolates 2, 27, 41, 57, 58, 67, 81, 97, 98, 103, and 104 were cultured from animals diagnosed with blastomycosis and provided by Dr. Al Legendre (University of Tennessee, Knoxville, TN). Isolate 57 (lymph node) and isolate 58 (skin lesion) were cultured from the same dog. The isolate Soil, was a kind gift from Dr. Alex Bakerspigel (Victoria Hospital Corp., London, Ontario, Canada) (10) and the human isolate Le was a kind gift from Dr. Norman Goodman (University of Kentucky School of Medicine, Lexington, KY). The organisms were grown at 25 °C (mycelial phase) or 37 °C (yeast phase) while shaking at 150 rpm in a chemically defined medium described by Johnson and Scalarone [11].

Southern blot analysis

Total genomic DNA from *B. dermatitidis* isolates was isolated by freezing with liquid nitrogen and grinding with a mortar and pestle as previously described [12]. *Eco*RI or *Bgl*II was used to digest 20 μg of *B. dermatitidis* DNA followed by separation on a 1.0% agarose gel. DNA was transferred to a nylon membrane (Micron Separations Inc., Westboro, MA) and UV fixed. The membranes were prehybridized at 68 °C in 5X Denhardt solution (0.1% Ficoll, 0.1% polyvinylpyrollidone, 0.1% bovine serum albumin (BSA) and 6X SSC (1X SSC is 0.15 M sodium chloride plus 0.15 M sodium citrate). Membranes were probed with a [α-^{32}P]dCTP-labeled (Oligolabeling Kit, Pharmacia, Inc., Piscataway, NJ) plasmid purified fragment of *bys1*. Blots were washed twice for 20 mm each in a series of increasingly stringent solutions: 10X SSC-1% SDS at room temperature, 1X SSC-1% SDS at 37 °, and 0.1X SSC-1% SDS at 68 °C. Washed Southern blots were then air dried and visualized by autoradiography.

Northern blot analysis

Total RNA was isolated from growing yeast or mycelial cultures of *B. dermatitidis* at various time points. The cells were frozen in liquid nitrogen and ground into a fine powder using a mortar and pestle and RNA isolated as described by Burg et al. [9] or by using the RNeasy kit (Qiagen). 10 μg of total RNA was treated with glyoxal and DMSO and electrophoresed in a 1.0% agarose gel using 10 mM sodium phosphate buffer, pH 7.0. The RNA was transferred to a nylon membrane (Micron Separations, Inc.) by capillary transfer. The membranes were prehybridized at 68 °C in Church buffer (1% BSA, 7% SDS, 0.5 M sodium phosphate (pH 7.0), and 1 mM EDTA). Membranes were probed with a radio-labeled plasmid purified fragment of *bys1* or *gapdh* in Church buffer at 68 °C overnight, then washed and visualized by autoradiography as described above. The relative absorbance of each Northern band was determined using the Gel Pro Analyzer (Media Cybernetics).

Cloning of B. dermatitidis *glyceraldehyde-3-phosphate dehydrogenase* (gapdh)

As a control for RNA lane loading of Northern blot experiments, Glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) from *B. burgdorferi* was cloned and used as a probe. Primers GAPDH 5′ (AGTACGACTCTACCCACGGC) and GAPDH 3′ (CGGTGTACGCGAGAATGCCC) were designed to conserved nucleotide residues based on an alignment of sequenced *gapdh* genes from multiple sources. SuperScript II (Life Technologies, Gaithersburg, MD) RNase H-Reverse transcriptase (200 Units) and Oligo-dT (500 μg/ml) were used to reverse transcribe 1.5 μg of *B. dermatitidis* total RNA as described by the manufacturer. cDNA from the first strand synthesis was

then used as template in a PCR reaction using a final concentration of 0.5 μM of GAPDH 5′ and GAPDH 3′ primers. Thermal cycling conditions consisted of a 95 °C soak for 3 min followed by 30 cycles of 97 °C for 15 sec, 60 °C for 1 min, and 72 °C for 2 min. The PCR fragment was cloned into the pCR 2.1 vector (Invitrogen Corp., Carlsbad, CA.). The insert was sequenced and confirmed to be *gapdh* by homology to other known *gapdh* genes.

Cloning and sequencing the bys1 *genes*

The *bys1* gene from isolates 2, 27, 41, 58, 81, 97, 103, 104, and Le were amplified in a 25 μl PCR mixture containing 10–30 ng/μl of genomic DNA and cycled in a DNA thermal cycler 480 (Perkin Elmer Cetus, Foster City, CA) using oligonucleotide primers bys 5′A (ATGCATCTCTCTTCTATA) and 3′UTR (GTTATCATAATACAGAGGC). Thermal cycling conditions consisted of 1 cycle of 95 °C for 3 min followed by 30 cycles of 97 °C for 15 sec, 49 °C for 1 min, 72 °C for 2 min and 1 cycle of 72 °C for 5 min. PCR products were cloned into pCR2.1 and sequenced.

Because the *bys1* gene from isolates 67, 98, and Soil didn't amplify with primers bys 5′A and 3′UTR, 3′Rapid Amplification of cDNA Ends (3′ RACE, Life Technologies) was used to amplify the *bys1* gene from the isolate Soil of *B. dermatitidis*. The procedure is the same as for RT-PCR (describe previously), except the Oligo-dT primer containing an adapter primer was used as the primer for reverse transcriptase elongation. After the RT procedure, primer 5′A and #1481 (adapter primer, CAUCAUCAUCAUGGCCACGCGTCGACTAGTAC) were used in a PCR reaction to amplify the *bys1* gene from the isolate Soil cDNA. The subsequent PCR fragment was cloned into pCR2.1 (Invitrogen) and sequenced. From this sequence, primer Soil UTR A (AAGAACTGAAGAGAAAATTAAC) was designed and used to amplify the *bys1* gene from isolates 67 and 98. The *bys1* nucleotide sequences have been deposited in GenBank and given the following accession numbers; isolate 2 (AF277079), isolate 27 (AF277082), isolate 41 (AF277083), isolate 57 (AF277084), isolate 58 (AF277085), isolate 81 (AF277086), isolate 97 (AF277087), isolate 98 (AF277088), isolate 103 (AF27708()), isolate 104 (AF277081), isolate Le (AF277089) and isolate soil (AF277090).

Table 1. B. dermatitidis isolates used in this study.

B. dermatitidis isolate	Cultured from	Geographic area
Class I		
#2	Dog	Tennessee
#27	Polar bear	Tennessee
#41	Cat	Tennessee
#57	Dog-LN*	Tennessee
#58	Dog-SK*	Tennessee
#81	Sea lion	Tennessee
#97	Dog	Minnesota
#103	Cat	Tennessee
#104	Cat	Tennessee
Le	Human	Kentucky
Class II		
#67	Dog	Minnesota
#98	Dog	Minnesota
Soil	Soil	Ontario, Canada

* Isolates #57 and #58 were isolated from the same infected dog. Isolate #57 was from the lymph node (LN) and isolate #58 was from a skin lesion (SK).

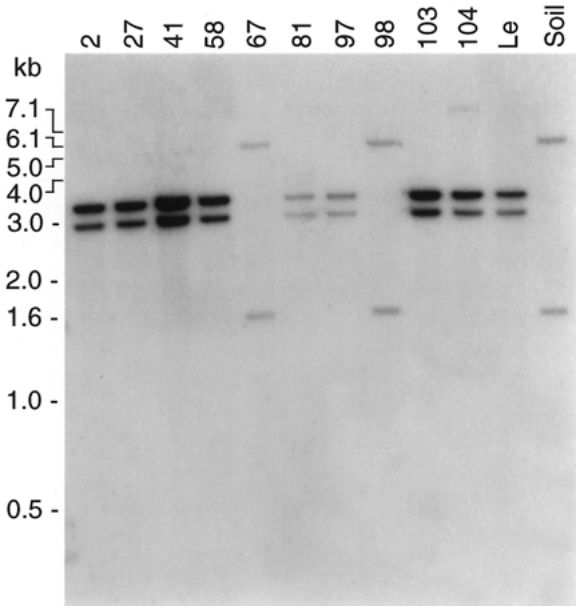

Figure 1. Demonstration of two different banding patterns of twelve different *B. dermatitidis* DNAs by Southern blot. Genomic DNA was isolated from *B. dermatitidis* isolates, digested with *Eco*RI and separated by agarose gel electrophoresis. DNA was transferred to a nylon membrane and hybridized with a *bys1* cDNA probe at 65 °C. *B. dermatitidis* isolates are indicated above the blot. The mobilities of size standards (in kb) are indicated on the left of the panel.

Results

Identification of bys1 *alleles in multiple* B. dermatitidis *isolates*

Twelve *B. dermatitidis* strains (Table 1) were subjected to Southern blot analysis using a *bys1* cDNA probe of the coding and untranslated regions (Figure 1). Two distinct banding patterns were observed among the twelve when *Eco*RI (Figure 1) or *Bgl*II (data not shown) were used to digest genomic DNA. DNA from isolates 2, 27, 41, 58, 81, 97, 103, 104, and Le digested with *Eco*RI produced genomic fragments of 3.17 kb and 2.81 kb and were grouped into class I. DNA from isolates 67, 98, and Soil had genomic fragments at 5.59 kb and 1.58 kb and were grouped into class II. The blots with *Eco*RI digested DNA were then stripped of full-length *bys1* probe and reprobed with a 372 bp 5′ fragment of *bys1*. The largest fragments from each banding pattern (class I-3.17 kb and class II-5.59 kb, Figure 1) hybridized with this probe (data not shown).

Amplification of the bys1 *gene using PCR*

PCR primers, bys 5′A and bys 3′UTR, were used to amplify a 830 bp fragment of the *bys1* gene that included the coding region (525 bp) and a portion of the 3′ untranslated region (305 bp). A 915 bp fragment was amplified from *B. dermatitidis* isolates 2, 21, 41, 57, 58, 81, 97, 103, 104, and Le all members of the class I *bys1* allele group (data not shown). In contrast, no PCR product was amplified from isolates 67, 98, and Soil, representatives of the class II isolates (data not shown). The two allelic groups of *B. dermatitidis* can be distinguished not only by Southern blot examination but by PCR as well. A slightly smaller PCR fragment was amplified with the *bys1* cDNA than from genomic DNA indicating that the *bys1* gene contains an intron (data not shown).

bsy1 *sequence comparisons*

PCR primers were designed to amplify the genomic regions of the two *bys1* alleles from which DNA sequence could be obtained and compared. The deduced amino acid sequence similarity of class I alleles was between 95.4%–100% similar and 73.7–74.9% similar to class II alleles which are 100% similar to each other (Table 2). The amino terminal end and several regions of the protein appear to be conserved between the two classes (Figure 2). The hydropathy plots of class I and II alleles deduced Bys1 proteins were very similar to each other (not shown). The deduced pI values for Bys1 class II (5.34) was slightly more basic than class I (4.35). The deduced Bys1 amino acid sequence from class I alleles contain two internal 34-amino-acid domains that share nine-amino-acid motifs as described previously[9]. The class II alleles do not have the two 34-amino acid domains but they do have a four-amino-acid motif that is shared with the class I allele (Figure 2a).

The 3′ untranslated region (UTR) of both allelic classes showed regions of homology and divergence (Figure 2b). Both classes have an 85 base intron 10 bases after the translation stop codon. The introns conform to the GT-AG rule for splicing junctions of nuclear genes in many eukaryotic cells [13]. In 80% of *Drosophila* introns, a branchpoint consensus adenosine is conserved as the 3′ splice signal and is also found in the *bys1* intron [13]. Interestingly 32 bases after the intron, both alleles contained within their 3′ UTR a consensus yeast transcription termination signal TAG . . . TAGT . . . TTT.

Furthermore, the 3′ UTR from class II is 51–53 bases shorter than class I and has only one of the presumptive AUUUA mRNA instability sequences, while class I has three [9], and class II also lacked the other presumptive instability sequence of a span of 9–11 U's. Because the bys 3′ UTR PCR primer is located upstream of the AAUAAA polyadenylation/cleavage sequence and one of the AUUUA mRNA instability sequences, these sequences from the class I genes couldn't be determined, although we assume they are present. From the sequence of the class II isolates, an AAUAAA polyadenylation/cleavage sequence is not found immediately upstream at the end of the 3′ UTR.

Northern blot analysis

Sequence analysis confirmed there were two different *bys1* alleles. As *bys1* transcription is strictly correlated with growth phase and culture temperature, we compared *bys1* mRNA levels between the two classes. Isolate #58 was previously characterized and was included as a control [9]. Class I, and II mRNA levels decreased when the temperature was lowered from 37 °C to 25 °C in the three isolates #58, Le, and Soil (Figure 3a–c). However subtle differences in *bys1* transcription were apparent among either class I and II members. After 24 hours, *bys1* mRNA transcription in Soil was no longer observed while class I isolates #58 and Le were still expressing *bys1* although at a

A.

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Isolate

58      MHLSSIFIGALTALISVVHAESVIAXRADLGSAFVFVYCNFSVALDIQAGETSTREILDG
soil    ......................V.......N....I....T.F..VE..GV...RT.N.
                              *       *    *    *    **   *   *  *

58      RSYDYHNEKYRRGGGDGVSLTLHHTDGPDSSNSETTFRYKLADDNSTVEYSLGNSGGNPF
soil    .NH..YH.P.GG.S........S..E.R.....R.......S.G....G..........
         **  **                 *       *       * *

58      AGHKITLKSSGDGCPNIEWPEGIPTGVSSGSCGSSENSILTLCPPGTSLDDFEDE
soil    E...L...P.D.R..R....D.............GA.L...M..LERPRQE....
            *   * *    *    *             *      *     * **
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B.

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Isolate

58      TAAAATTAAAATGgtatgatgtgactt--ctctgtctttttttttcactcgtgatttgatg
soil    ......................t..gtc................t-..a.........a

58      agtatttt--gagtttgatcatgctaataattgagagataagAAAATGAAACCTTTTTGT
soil    .......gag.g.....---.......................................

58      TTCATGATTAGACATAGTCACCTGTCAACTTCTCTTAACATTAGTTGAATTTCTCAATGT
soil    ........................C..............T......A.....T.....T.

58      GAGATTATGAA-GAGATCAGGCAATCAGTGACATAATTTAA-------------GTTAGA
soil    ...........A..A....T.........AG........G.CTCATCTTATTGA..C.T.

58      GCTCATGCTAATAAGCATTAAAACATTGTTTGAGATATTTTATCAATGTATTTAATTATT
soil    TGA............G..C...G..................G.G............C...

58      AAAGACAGATGTTAATTTTCTCTCTTCTATCTTTATTAATTTTTTTTTTCATTAACTCTT
soil    .....T.-.A-------------.A.G...---.G........------.------....

58      ATTCTCTTATTTTATATATAAAGTATGCCTCTGTATTATGATAAC
soil    CAGT....-----------------------------------
```

Figure 2. (a) Amino acid alignment of the Bys1 gene from a representative *B. dermatitidis* isolate of allelic group I (#58) and II (Soil). The two internal 34-amino-acid domains that share a nine-amino-acid motif are in bold type. Asterisks are located below amino acids that share similarities. (b) Nucleotide sequence alignment of the 3′ UTR of the *bys1* gene from a representative *B. dermatitidis* isolate of allelic group I (#58) and II (Soil). The 85 nucleotide intron is in lower case. The putative 3′ intron splice variant nucleotide sequence is in bold and italic type. A yeast transcription termination consensus sequence is underlined over the nucleotides. The putative mRNA instability sequences are in bold type.

lesser amount. Because isolates #58 and Le were still expressing *bys1* mRNA after 24 hours, RNA was extracted from isolate Le at 24 hour intervals for 5 days and assayed for *bys1* transcripts. A decrease in Le bys1 mRNA transcript was observed after 24 hours but then the levels of transcription increased to time point 72 hours before decreasing to very low levels (Figure 4).

To complete the dimorphic life cycle of *B. dermatitidis*, *bys1* transcription was also examined during a phase/temperature shift from mycelial (25 °C) to yeast (37 °C). Class I isolates #58 and Le *bys1* mRNA transcripts were observed after 24 and 48 hours respectively, while *bys1* mRNA was not measurable from the Soil isolate (Figure 5a–c). A Northern analysis of *bys1* mRNA from the isolate Soil after 5 days of growth at 37 °C indicated that *bys1* mRNA transcript was still immeasurable (Figure 6).

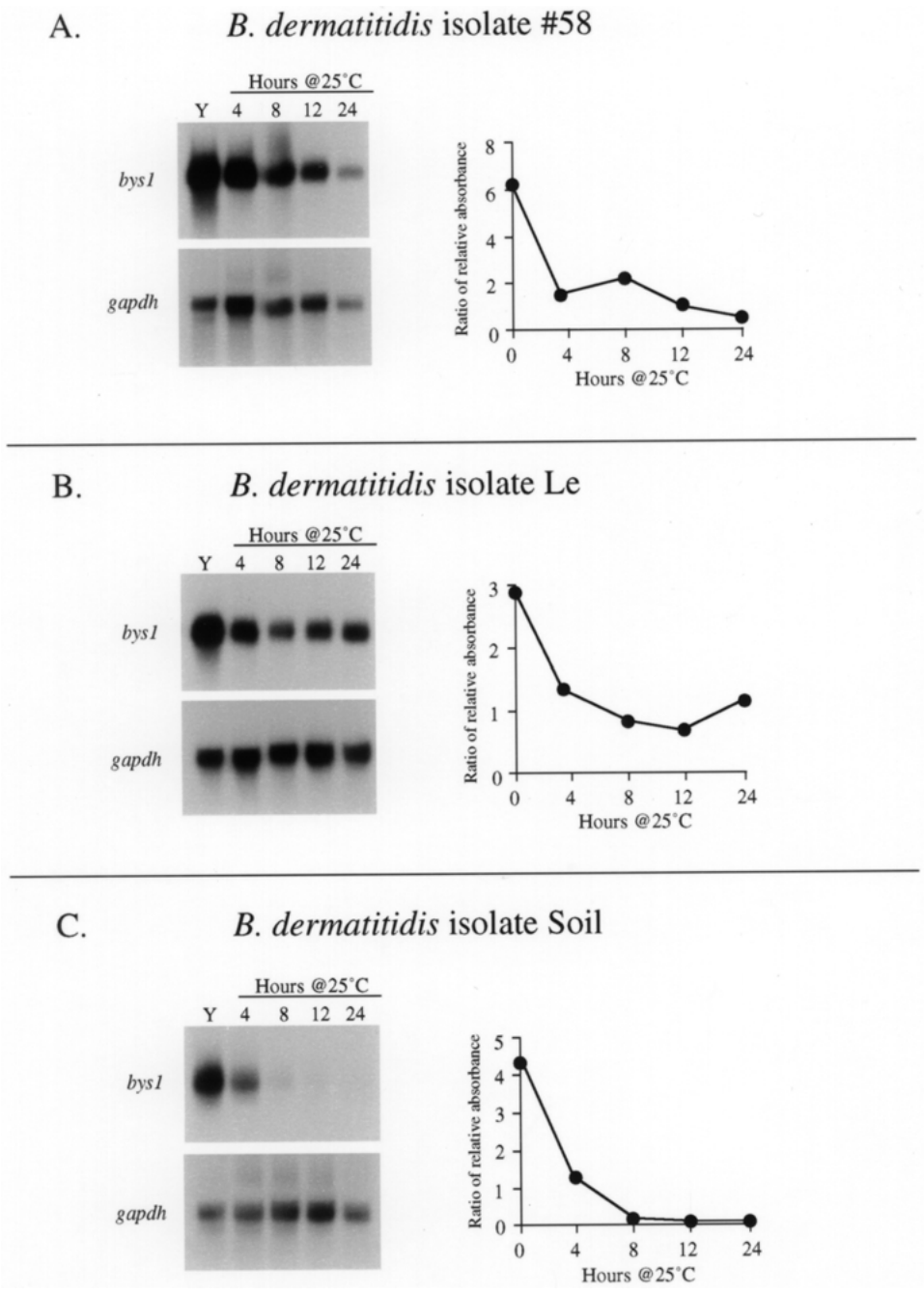

Figure 3. Analysis of *bys1* transcript from *B. dermatitidis* isolates. Total RNA was extracted from *B. dermatitidis* isolates #58 (a), Le (b), and Soil (c) growing at 37 °C (Y) and then 4, 8, 12 and 24 hours after the temperature was lowered to 25 °C. The RNA was separated by electrophoresis, transferred to a nylon membrane and hybridized with a probe made from the *bys1* gene of isolate #58. The blots were then allowed to decay and hybridized with a *gapdh* probe to assure equal loading of the RNA in each lane. The relative absorbance of each *bys1* band and its corresponding *gapdh* band were determined using the Gel Pro Analyzer system. The right panel is a graph of the relative absorbance of the *bys1* band divided by the relative absorbance of the *gapdh* band.

Table 2. Identity of the deduced amino acid sequences of Bys1 from multiple isolates.

Isolate	2	27	41	57	58	81	97	103	104	Le	67
2	–										
27	97.1	–									
41	97.1	98.9	–								
57	97.1	98.9	98.9	–							
58	97.9	99.4	99.4	99.4	–						
81	96.6	96.0	96.0	96.0	96.6	–					
97	97.1	98.9	98.9	98.9	99.4	96.0	–				
103	96.6	98.3	98.3	98.3	98.9	95.4	98.3	–			
104	97.7	99.4	99.4	99.4	100	96.6	99.4	98.4	–		
Le	97.7	99.4	99.4	99.4	100	96.6	99.4	98.9	100	–	
67*	74.9	72.7	73.7	73.7	74.3	74.9	74.3	73.7	74.3	74.3	–

Comparisons were performed using Clustal V program with the following alignment parameters: fixed gap penalty, 10; floating gap penalty, 10; protein weight matrix, PAM250. Values indicate percent similarity.

* Isolate #98 and Soil deduced amino acid sequences are identical to isolate #67.

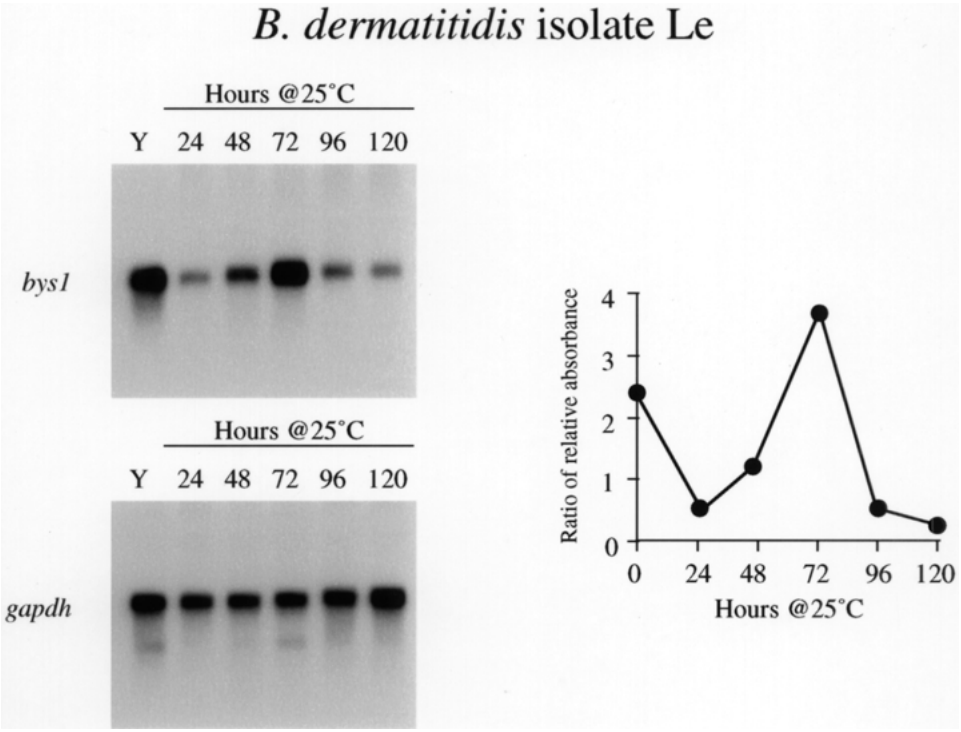

Figure 4. Analysis of *bys1* transcript from *B. dermatitidis* isolate Le. Total RNA was extracted from *B. dermatitidis* isolate Le growing at 37 °C (Y) and then 24, 48, 72, 96 and 120 hours after the temperature was lowered to 25 °C. The RNA was separated by electrophoresis, transferred to a nylon membrane and hybridized with a probe made from the *bys1* gene of isolate #58. The blots were then allowed to decay and hybridized with a *gapdh* probe to assure equal loading of the RNA in each lane. The relative absorbance of each *bys1* band and its corresponding gapdh band were determined using the Gel Pro Analyzer system. The right panel is a graph of the relative absorbance of the *bys1* band divided by the relative absorbance of the *gapdh* band.

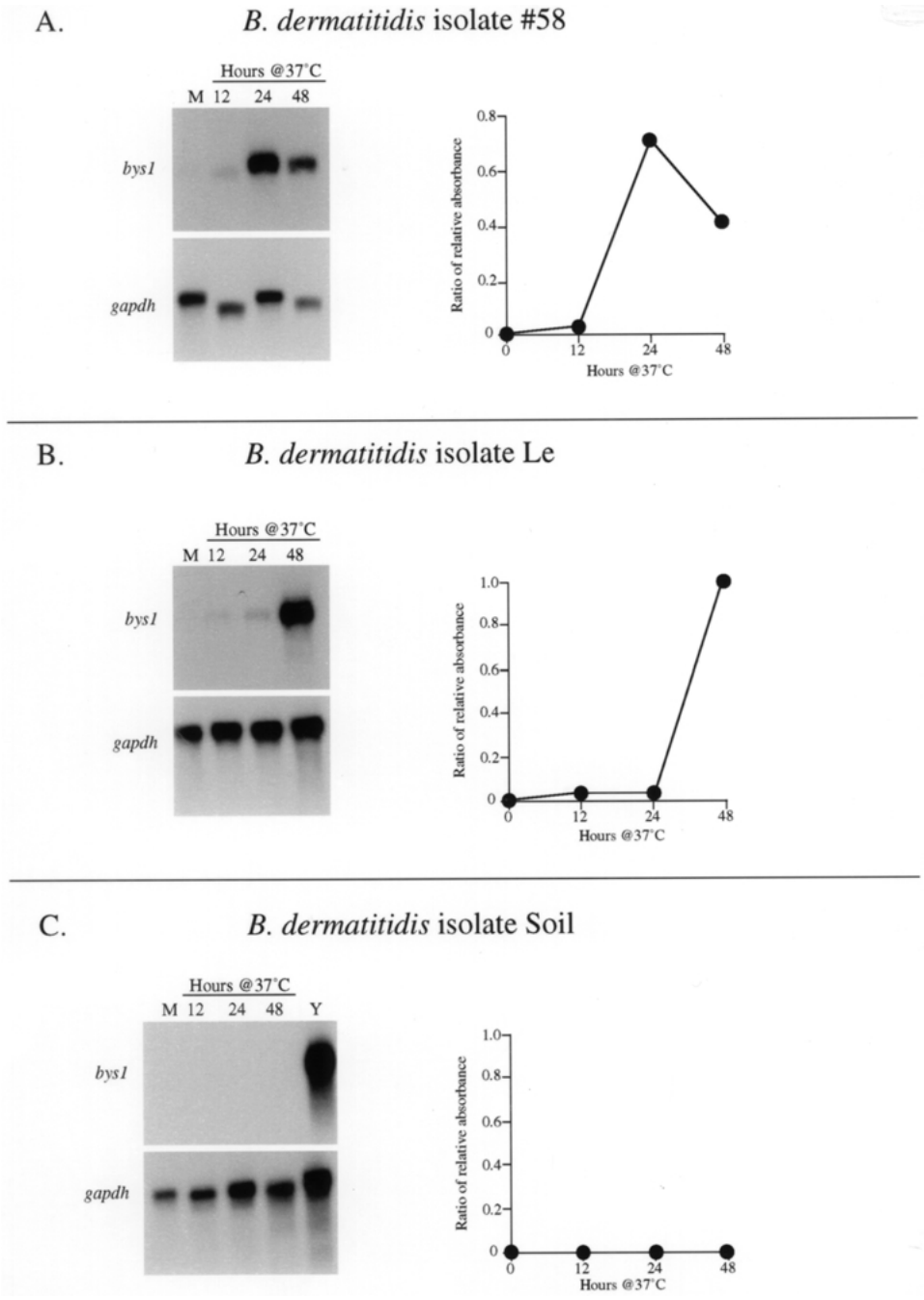

Figure 5. Analysis of *bys1* transcript from *B. dermatitidis* isolates. Total RNA was extracted from *B. dermatitidis* isolates #58 (A), Le (B), and Soil (C) growing at 25 °C (M) and then 12, 24 and 48 hours after the temperature was raised to 37 °C. RNA from *B. dermatitidis* isolate #58 yeast phase (Y) was included as a positive control on the membrane containing RNA from the isolate Soil (C). The RNA was separated by electrophoresis, transferred to a nylon membrane and hybridised with a probe made from the *bys1* gene of isolate #58. The blots were then allowed to decay and hybridized with a *gapdh* probe to assure equal loading of the RNA in each lane. The relative absorbance of each *bys1* band and its corresponding *gapdh* band were determined using the Gel Pro Analyzer system. The right panel is a graph of the relative absorbance of the *bys1* band divided by the relative absorbance of the *gapdh* band.

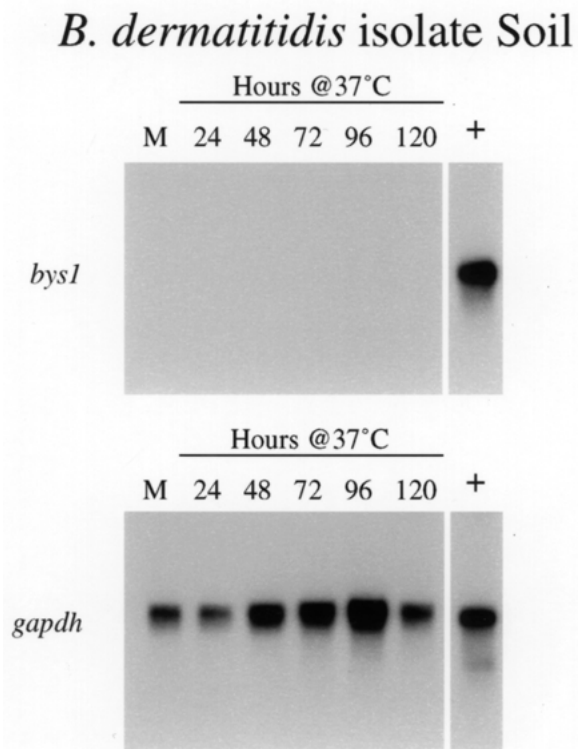

Figure 6. Analysis of *bys1* transcript from *B. dermatitidis* isolates. Total RNA was extracted from *B. dermatitidis* isolate Soil growing at 25 °C (M) and then 24, 48, 72, 96, and 120 hours after the temperature was raised to 37 °C. RNA from *B. dermatitidis* isolate #58 yeast phase (+) was included as a positive control. The RNA was separated by electrophoresis, transferred to a nylon membrane and hybridized with a probe made from the *bys1* gene of isolate #58. The blots were then allowed to decay and hybridized with a *gapdh* probe to assure equal loading of the RNA in each lane. The relative absorbance of each *bys1* band and its corresponding *gapdh* band were determined using the Gel Pro Analyzer system. The right panel is a graph of the relative absorbance of the *bys1* band divided by the relative absorbance of the *gapdh* band.

Discussion

We have shown that the *bys1* gene is found in all isolates of *B. dermatitidis* examined and that it exists in at least two allelic forms. Genetic diversity of *B. dermatitidis* has been described previously using several different probes in Southern blots [14]. Fraser et al [15] used an 8.4-kb rDNA repeat clone [14] to probe 4 clinical isolates of *B. dermatitidis* and concluded that the 4 isolates could be grouped into three classes. In a larger study, Yates-Sulata et al. [12] were able to group 19 clinical *B. dermatitidis* isolates from Arkansas into three classes by restriction fragment length polymorphisms using *H. capsulatum* mtDNA and rDNA probes [14] and by PCR-based random amplified polymorphic DNA assay. Interestingly, both studies classified the isolates into three classes, although it cannot be determined if the three classes from the two studies are the same. Yates-Sulata et al [12] conduded that a variety of factors such as genetic drift, exchange between genomes following sexual mating, and unique selection pressures impacting on the organism in various environmental niches, may influence the genetic structure of *B. dermatitidis* populations [12]. These sources of genetic diversity may also explain the two classes of *B. dermatitidis* that we observed. The apparently distinct geographic distribution would seem to favor the possibility that selective pressure exerted by different environmental niches resulted in two *bys1* alleles.

In a recent study [16], fifty-nine *B. dermatitidis* isolates were studied by a PCR-based typing system, which used restriction fragment analysis and PCR fingerprinting to differentiate the isolates into 3 major groups. An interesting observation was that all 16 North American isolates in group A were from upper midwestern United States or Canada with 8 in group B and 7 in group C, whereas none of 20 isolates from the southeastern United States were in group A, but in either group B or C. We observed a geographic distribution similar to what MacCullough et al. [16] described although only two classes or groups were defined. Only isolates from the midwestern United States and Canada made up class II whereas none of the 8 isolates from the southeastern United States were in class II. The soil isolate from Canada was the only sample that was redundant between our study and those by MacCullough et al. [16]. Interestingly, this isolate was grouped or classified into isolates from midwestern United States or Canada in these studies.

Isolates 57 and 58 were isolated from the same animal but at two separate sites, a lymph node and a skin lesion, respectively. The PCR amplification and Southern blot data indicate that they belong to the same class. There was one nucleotide difference in 912 bases sequence that caused an amino acid change from the deduced protein sequence between the two isolates. From these results, the same isolate was able to cause multiple lesions in a single infected animal.

Transcription of both class I and class II *bys1* alleles apparently decreases in the mycelial growth phase. However, it is clear that the two alleles are regulated differently. While the *bys1* gene in class I alleles is transcribed 24–48 hours after a temperature up shift from 25 °C to 37 °C, the *bys1* gene from a class II isolate, Soil, isn't transcribed even 120 hours

after the culture temperature was increased from 25 °C to 37 °C. Transcription of *bys1* mRNA from the class II isolate Soil is terminated 8 hours after a decrease in temperature from 37 °C to 25 °C while the two class one isolates are still transcribing *bys1* mRNA after 120 hours [9]. From this, we assume that the Bys1 protein probably doesn't function in the transition from one phase to the other but performs some function in the yeast phase. Currently, there is no other protein in the database with significant protein sequence homology to Bys1. A Y-chromosome repeat sequence in *Bubalas bubalis* (water buffalo) named BBYS1 claims to have the same amino acid repeat motif as Bys1 but shares only 8.6% identity and 12.0% similarity.

AU-rich elements (ARE) in the 3′UTR of many mammalian mRNA confers message instability and is often found within a U-rich region of the mRNA [18-20]. The 3′UTR of isolate #58 has 3 AREs [9] but the class II alleles have only one. This suggests that the mRNA from class II is more instable than class I. While more *bys1* transcript appears sooner and persists longer in the Northern blots of class I isolates #58 and Le than class II isolate Soil, we suggest that mRNA stability doesn't account for the differences in *bys1* mRNA levels but that the *bys1* mRNA is transcriptionally regulated.

In conclusion, we have identified two alleles of *bys1* from the most diversified group of *B. dermatitidis* isolates studied to date. We have defined two classes of *B. dermatitidis* by molecular typing of the *bys1* gene. The differences in protein sequence and gene expression between the two alleles may reflect adaptive changes in *B. dermatitidis* to different environments and adds to previous data [16], which suggests there is geographic diversity among *B. dermatitidis* isolates. We are continuing to investigate the relevance of the two alleles of *bys1*, the differences in their apparent gene transcriptional control, and the function of the Bys1 protein.

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