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NOTE

Simple Identification of *veA1* Mutation in *Aspergillus nidulans*

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The *veA* gene plays an important role in development of a homothallic filamentous fungus *Aspergillus nidulans*. The *veA1* phenotype can be difficult to distinguish from the wild-type *veA*. Despite the importance of the *veA* allele, no efficient identification method has been reported besides DNA sequencing. Here, we present simple physiological and molecular biological ways to distinguish between the *veA* wild-type and *veA1* allele. The novel approaches, which involve incubation in the presence of oxalic acid, polymerase chain reaction using double mismatched primers, and *Bst*XI enzyme digestion, are simpler, faster and more cost-efficient than genome sequencing.

***Keywords*:** *Aspergillus nidulans*, *veA*, *veA1* mutation, discrimination

The *veA* gene encodes a key positive regulator of sexual development of *Aspergillus nidulans* (Kim *et al*., 2002). The VeA polypeptide consists of 573 amino acids and harbors a bipartite nuclear localization signal (NLS) in the N terminus (Stinnett *et al.*, 2007). VeA is also involved in red-light repression of sexual development and acts downstream of FphA (Blumenstein *et al.*, 2005). In the dark, VeA is located mainly in the nuclei, while under light VeA is found abundantly in the cytoplasm, indicating that the migration of VeA to the nucleus is light-dependent (Stinnett *et al.*, 2007). An allelic mutation of the *veA* gene, *veA1*, causes delayed and reduced production of fruiting bodies (Champe *et al.*, 1981). A G → T transversion in the translation initiation codon of the wild-type *veA* gene is the basis of the *veA1* mutation, which results in the use of ATG at the 37th codon as a translation initiation codon (Kim *et al.*, 2002). Due to the mutation, the VeA1 mutant protein lacks the 36 amino acid at the N-terminus. Thus, the VeA1 polypeptide is found predominantly in the cytoplasm, which causes VeA1 to be not functional and fails to regulate the genes required for sexual development (Stinnett *et al.*, 2007).

The *veA1* mutant obtained by Käfer (1965) was by happenstance during the introduction of genetic markers in *A. nidulans* (Käfer, personal communication). Most laboratory strains carry the *veA1* mutation, which makes the genetic study of sexual development difficult. Although a number of mutants defective in sexual development have been isolated using the *veA* wild-type strain (Han *et al*., 1990; Min *et al*., 2007), it is still necessary to identify the *veA* allele type after genetic crossing with test strains, because almost every test strain carries the *veA1* mutation. However, it is not easy to discriminate the *veA* wild-type from *veA1* mutation precisely only on the basis of colony morphology. Nucleotide sequencing of the mutated region can be the most precise way to verify the *veA* allele, but it is the most time-consuming and expensive way. To overcome these hurdles of sequencing, here we present several physiological and molecular biological approaches, which more swiftly and economically identify the *veA* allele than genome sequencing.

As shown in Fig. 1A, asexual spores developed preferentially in the *veA1* strain compared to the *veA* wild-type. However, the morphological traits of the colonies were not

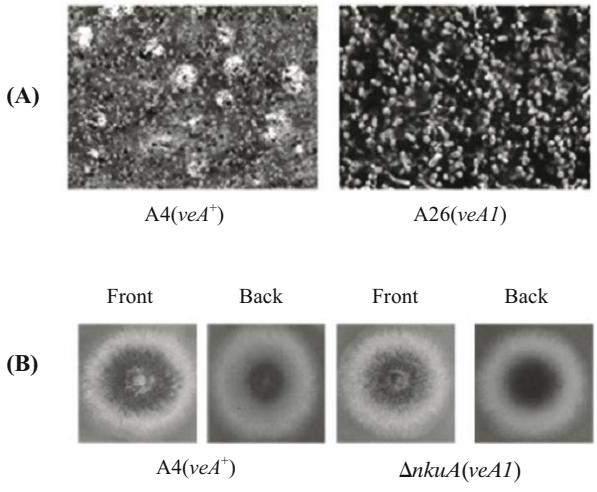

Fig. 1. Phenotypic difference between wild-type *veA* and *veA1* mutant in cultures from plate (A) or point inoculation (B).

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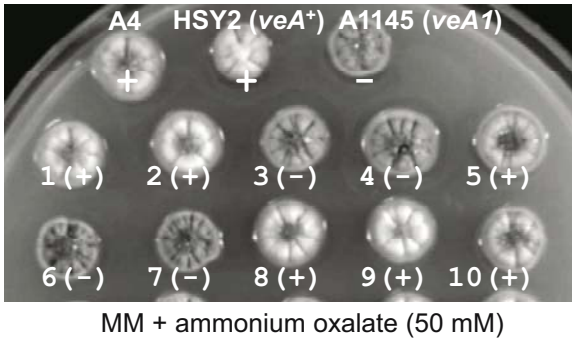

Fig. 2. Morphological difference of colonies of the wild-type *veA* and a *veA1* mutant grown on the minimal medium containing 50 mM ammonium oxalate. The wild-type does not produce conidia and produces bright colonies, while the *veA1* mutant normally develops conidia, and so forms dark colonies

precisely distinguishable when the strains were cultured from point inoculation (Fig. 1B). Oxalic acid, which is an inhibitor of the tricarboxylic acid (TCA) cycle inhibitor, inhibits the conidiation of the *veA* wild-type, but not the *veA1* mutant strain, in a dose-dependent manner (Han *et al*., 2003). Taking the advantage of this property, we applied ammonium oxalate into the culture medium to isolate *veA* wild-type segregants obtained by a genetic cross between veA^+ and *veA1* strains. In this experiment, we used FGSC A1145 strain (*pyrG89*; *pyroA4*, Δ*nku::argB*; *riboB2*; *veA1*; kindly donated by Dr. Michael Hynes, University of Melbourne). The strain harbors the Δ*nkuA* mutation causing reduction of non-homologous recombination frequency and, hence, enhances the gene deletion frequency by homologous recombination (Nayak *et al*., 2006). Since the strain contains the *veA1* allele, it is inadequate to use for the screening of mutants having defects of sexual development. Thus, we genetically crossed A1145 with a veA^+ strain, HSY2 (*anA1*; Δ*argB*::*trpC*; veA^+; Kim *et al*., 2009), and isolated veA^+, Δ*nkuA* recombinants from the meiotic progenies on a minimal medium supplemented with 50 mM ammonium oxalate (Fig. 2). The minimal medium for *A. nidulans* with appropriate supplements was prepared as described by Han *et al*. (2001) and FGSC A4 strain, obtained from Fungal Genetics Stock Center, was also used for verification of wild-type *veA* allele. Since the *veA* wild-type strain did not produce conidia on oxalate-containing medium, the wild-type colonies could be easily distinguished from *veA1* strains, which developed conidia normally. Among 10 $argB^+$ progenies carrying Δ*nkuA* mutation, six strains (samples 1, 2, 5, 8, 9, and 10) were visually identified as veA^+ strains (Fig. 2).

Since just a single nucleotide change occurred in *veA1*, a primer containing the single mismatched sequence would not be sufficient to verify the mutation by conventional polymerase chain reaction (PCR). The extreme 3′-end of a primer is important for successful PCR and a mismatched 3′-end of primer significantly affects the reaction (Kwok *et al*., 1990). These previous observations prompted our hypothesis that a single mismatch primer for the wild-type allele can produce the PCR product but a double mismatched primer for *veA1* allele will not. The aforementioned transversion mutation in *veA1* generates an ATT codon instead of the wild-type ATG codon. Presently, we designed a forward primer whose 3′-end was AGG and performed PCR using this double mismatched primer set (Fig. 3A). For the PCR experiment, ExPrime Taq polymerase from GeNet Bio (Korea) was used. *A. nidulans* genomic DNA was extracted as described previously (Han *et al*., 2001). The double mismatched forward primer 5′-TG TGT TAT CCC ATC AAG AGG-3′ and reverse primer 5′-TCT CCG CGC CGT CTC ATC-3′ were used as the primer set for this double mismatched PCR. The PCR conditions were 30 cycles of 30 sec at 94°C, 30 sec at 55°C, 1 min at 72°C with 30 cycles. PCR was successful only in samples 1, 2, 5, 8, 9, and 10; no band was detected in samples 3, 4, 6, and 7 (Fig. 3B). The results exactly mirrored the results pertaining to colony morphology using oxalic acid.

Although PCR using double mismatched primer amplified the wild-type *veA* allele, it could be argued that the negative result merely reflected the failure of an ordinary PCR experiment. To overcome this drawback, a more obvious and precise

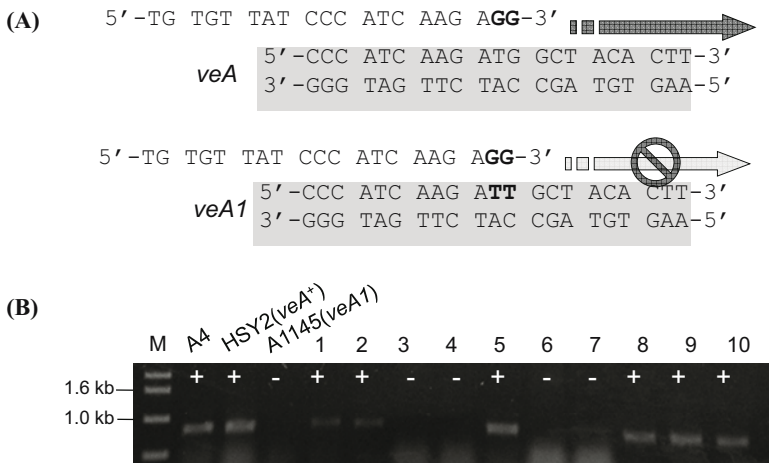

Fig. 3. Schematic diagram of PCR with double mismatched primer (A) and PCR amplification result using double mismatched primer (B). Nucleic acid pairs in the gray box represent genomic DNA sequence of either the wild-type *veA* or the *veA1* allele. Single strand nucleic acid sequence shows the double mismatched primer and bold characters represent mismatched region. The arrow means amplification of PCR.

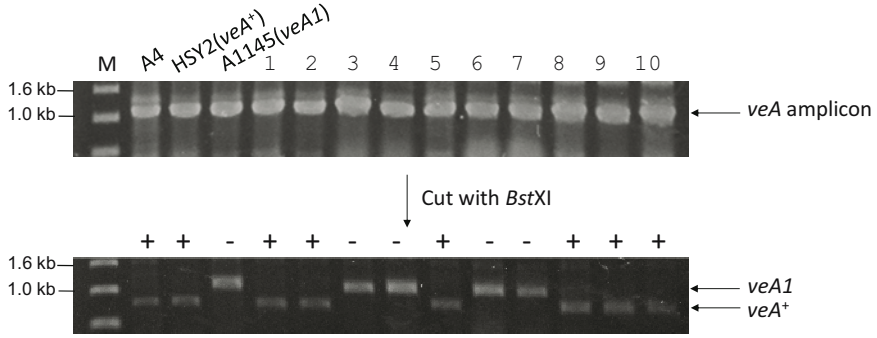

Fig. 4. Result of PCR amplification of genomic DNA region of the veA allele followed by *Bst*XI restriction enzyme digestion. The PCR amplicon from veA1 mutant allele was not digested by *Bst*XI, resulting in a longer band than the wild-type veA amplicon after *Bst*XI digestion.

method was designed using restriction enzyme digestion. This tact took advantage of the fact that the wild-type *veA* allele possesses a *Bst*XI enzyme site (5′-CCANNNNNNTGG-3′) at the region of the start codon (Fig. 4). The *veA1* mutation occurring at this abrogates the *Bst*XI recognition site. Hence, checking the availability of the enzyme site in the *veA* region can clearly discriminate the *veA* wild-type allele from the *veA1* mutant allele. To this end, PCR forward primer 5′-AGC CCA TCC AGC CCA TCT-3′ and reverse primer 5′-TCT CCG CGC CGT CTC ATC-3′ were designedto amplify the 1,061 bp DNA fragment containing *veA* start codon in the middle, enabling to yield 791 bp and 270 bp DNA fragments when wild-type *veA* allele is digested by *Bst*XI (Fig. 4A). The 10 progenies tested the phenotype on oxalate medium were also used for the *Bst*XI digestion analysis. The PCR products by using same condition described earlier were obtained from all genomic DNAs from recombinants as well as control strains (Fig. 4), and were digested with *Bst*XI. PCR products of wild-type control (FGSC A4, HSY2) were digested and produced 791 bp and 270 bp DNA fragments (Fig. 4). As expected, *Bst*XI could not digest the PCR products from the *veA1* control strain (A1145) and four *veA1* progenies. This result coincided exactly with that obtained by phenotypic analysis on oxalate medium and the PCR results using double mismatched primer. The unity in the three methods for identifying the *veA* and *veA1* alleles provided confirmation that a combination of the methods allows the accurate identification of the *veA* allele more quickly and economically than using genomic DNA sequencing.

We tried to select a *veA*$^{+}$, Δ*nkuA* recombinant through meiotic segregation in order to use it as a host strain for isolation of deletion mutants of genes involved in sexual development. We successfully isolated recombinants and confirmed the wild-type *veA* genotype using several useful techniques, which we anticipate will be useful for many other strains whose developmental phenotypes are disturbed by *veA1* mutation.

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