F328 Structural and functional characterization of the *veA* gene required for sexual development in *Aspergillus nidulans*

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The gene complementing the *veA1* mutation was confirmed to be the *veA* gene. The nucleotide sequences of the cDNA isolated as well as its genomic DNA were determined, showing that there is one open reading frame (ORF) possibly coding for a 573 amino acid polypeptide in the gene interrupted by two introns. No similarities in the amino acid sequence as well as in the nucleotide sequence were found to anyone currently available in GenBank and SWISS-Prot, respectively. It had a putative PEST region that contains large amounts of proline (P), glutamic acid (E), serine (S), and threonine (T), and a nuclear localization signal, PKRARAC. The nucleotide sequence of the *veA1* mutant gene was also determined and was differed by one nucleotide from a wild type one. The mutant ORF seemed to have the 37th methionine codon of the wild type one as a new initiation codon. The expression of the gene started from 14 hours after inoculation of conidia, and continued constitutively with the increased level throughout sexual and asexual developments, suggesting that it may act from the early developmental stage. A null mutant of the gene constructed by replacing a part of its ORF with the *argB* gene never entered sexual development even under conditions where sexual development preferentially occurs. The induced expression of the gene in a *veA1* mutant resulted in the formation of sexual structures even under the condition where very little sexual structures in wild type strains are formed. These results indicated that the gene is a positive regulator for sexual development, although a possibility that it also can be a negative one for asexual development can not be ruled out. Consistent with this is that it had an ability to trans-activate a reporter gene in *Saccharomyces cerevisiae* when a DNA binding domain of Escherichia coli LexA protein was provided in its N-terminus to produce a hybrid protein, LexA-VeA.

F329 Expression of the *INU2* Gene for an Endoinulinase of *Aspergillus ficuum* in *Saccharomyces cerevisiae*

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The *INU2* gene encoding an endoinulinase of *Aspergillus ficuum* was expressed by the *Kluyveromyces marxianus INU1* promoter in a *SUC2*-deleted *Saccharomyces cerevisiae* to produce the endoinulinase free of an exoinulinase and an extracellular invertase in the culture medium. A recombinant yeast strain produced the enzyme at the concentration of 30 U/ml into a culture medium upon induction by fructose. The amount of enzyme in one ml can hydrolyze 180 mg inulin in one hour. The *INU2* product seemed to be processed properly in yeast and secreted well into a medium enough to produce fructo-oligosaccharide from inulin by direct use of the culture broth as an enzyme source. When the recombinant yeast strain was cultured in a medium prepared by boiling 60 g of wet chicory root particles in 100 distilled water for 15 min, the eluted inulin was completely hydrolyzed during overnight culture. Therefore, the yeast strain could be used for manufacturing a non-alcoholic beverage of chicory. The molecular weight of the secreted enzyme was 67 kDa as measured on Western blot by using polyclonal anti-endoinulinase antibodies, which was larger by 1 - 3 kDa than those of the purified enzymes.