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Cloning and Sequencing of the Genomic DNA Encoding Putative Mitogen-Activated Protein Kinase from a Phytopathogenic Fungus Colletotrichum gloeosporioides

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정제적으로 유용한 벼, 채소 등의 작물에 가장 많은 손실을 끼치는 것은 Magnaporthe grisea 등과 같은 식물병원성 곰팡이들이다. 이와 같은 병원성곰팡이에 의한 식물의 감염에 MAP kinase를 이용하는 신호전달계가 근본적으로 필요한 것으로 밝혀졌다. 그러므로 MAP kinase가 제거된 돌인변이주는 식물질병을 유발하지 않은 것으로 보고되었다. Colletotrichum gloeosporioides의 MAP kinase 유전자를 클로닝하기 위해 yeast MAP kinase인 FUS3, KSS1, HOG1과 Magnaporthe grisea의 MAP kinase인 PMK, Fusarium solani의 FsMAPK의 conserved amino acid로 degenerated primer를 제작하여 PCR cloning에 이용하였다. 약 500 bp의 MAP kinase의 단편을 cloning하였으며 이는 M. grisea와 염기 서열상 93%, 아미노산 서열상 93%를 F. solani의 FsMAPK와 82%, 68% 상동성을 보였다. 또한 genomic library를 작성 colony hybridization을 시행한 결과 약 5.0 - 5.5 kb의 유전자를 가진 candidates를 얻었다. 이 candidates로부터 PCR를 행하여 sequencing한 결과 위의 MAP kinase 유전자 500 bp DNA fragment를 포함하고 있었다.

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F309 A Gene Responsible for Paraquat-Sensitivity of Streptomyces coelicolor Encodes a DNA-Binding Protein Similar to NfxB of Pseudomonas aeruginosa

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Differentiation of Streptomyces coelicolor A3(2) was arrested by moderate amount of paraquat (methyl viologen), a superoxide-generating agent, with no apparent inhibition of cell growth. We isolated three mutants whose sporulation and antibiotic production was not affected by paraquat. These mutations were mapped to a single locus near argA1 at about 1 o'clock on the genetic map. We isolated a novel gene (pqr; paraquat-resistant) required for paraquat-resistant differentiation from one pqr mutant as a dominant allele to the wild type allele. The pqr gene consists of two genes (pqrA and pqrB). The pqrA gene specifies a protein containing a DNA-binding motif and the pqr mutation was located within its gene product, which generates an amino acid substitution (arginine to glutamine). The pqrB gene encodes a putative membrane protein probably involved in membrane associated energy-driven efflux of drugs including paraquat. Both wild type and mutant PqrA proteins specifically bind to an inverted repeat structure (PRE) overlapping with the putative pqrAB promoter as demonstrated by gel retardation. These results suggested that pqrA encodes a DNA-binding protein which might be involved in regulating the expression of pqrAB operon.