본 실험실에서 밝힌 *Colletotrichum gloeosporioides*의 MAP Kinase인 CGK1의 염기서열를 이용하여 제작한 primer를 사용하였다. Nucleotide sequencing 결과 PCR product가 MAP kinase 유전자임을 확인하였다.

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Characterization of Genomic DNA Encoding Full Length of Mitogen-Activated Protein Kinase, CGK1 from *Colletotrichum gloeosporioides*

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*Colletotrichum gloeosporioides*의 MAP kinase 유전자를 클로닝하기 위해 yeast MAP kinase인 FUS3, KSS1, HOG1과 *Magnaporthe grisea*의 MAP kinase인 PMK1, *Fusarium solani*의 FsMAPK의 conserved amino acid로 degenerated primer를 제작하여 PCR cloning에 이용하였다. 약 500 bp의 MAP kinase의 단편을 cloning하여 이를 probe로 이용, Southern blotting, colony hybridization을 시행하였고 10여개의 candidates를 확보하였다. 이를 Gene bank에 CGK1으로 등록하였다 (Entry ID: 20000731153447.87708). 또한 *C. gloeosporioides*의 약 1.1 Kb 크기의 cDNA를 cloning였으며, genomic DNA와 비교하여 약 60여개의 nucleotide를 가진 3개의 intron 지역을 밝혔다. CGK1을 overexpression한 결과 43kDa의 protein이 과대발현되었다. *C. gloeosporioides*의 MAP kinase인 CGK1의 상동성은 *M. grisea*의 PMK1과 염기 서열상 84%, 아미노산 서열상 95%를, *C. lagenarium*의 CMK1와 85%, 99%, F. solani의 FsMAPK와 84%, 58% 상동성을 보였다. 그러나 Yeast의 Fus3와 KSS1과의 아미노산 상동성은 58% 61%를 보였다.

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*Enterobacter aerogenes*의 C-P Lyase (*Phn*) Operon 구조분석

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Phosphonate (Pn)은 carbon-phosphorus (C-P) bond를 포함하는 거대분자로서 phosphate가 제한된 환경에서 미생물에 의해 이용된다. *Escherichia coli* 의 경우 C-P lyase pathway는 C-P lyase (*phn*) operon에의해서 이루어짐이 밝혀졌고 장내세균인 *Enterbacter aerogenes*에서도 *phn* operon이 존재함이 밝혀졌다(Lee *et al.*, J. Bacteriol., 174: 2501-2510, 1992). *Enterbacter aerogenes*의 *phn* operon을 mini-mu phage를 이용하여 cloning 하였고 이의 염기서열을 분석한 결과 *pho* box, -10 region, RBS site가 *phn*F의 5'말단에 있으며 *phn*FGHIJKLMNP로 구성되어 있음을 알수 있었다. *Escherichia coli*의 *phn* operon과는 아미노산서열이 평균 82.6%의 상동성을 지니며 *phn*J와 *phn*K가 92%로 가장 높았고 *phn*G와 *phn*N이 71%로 가장 낮은 상동성을 보였다.

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Enterbacter aerogenes 의 C-P Lyase (*Phn*) Operon Promoter의 기능규명

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*Enterbacter aerogenes*는 C-P direct compound를 이용하는 기작으로 phosphonatase pathway와 C-P lyase pathway를 가지고 있다. 그중 C-P lyase pathway에 관여하는 *phn* operon은 10개의 gene (*phn*FGHIJKLMNP)으로 구성되어 있고 regulatory gene 인 *phn*F 의 upstream쪽에서 *pho* box와 -10 region 그리고 RBS가 있음을 염기서열분석으로 확인하였으나 그 조절기작은 불분명하다. *phn* Operon promoter region의 기능을 확인하기 위하여 PCR을 통해서 cloning을 하였고 CAT assay를 시행한 결과, *Enterbacter aerogenes*에서도 *phn* operon의 transcriptional regulation은 PhoB protein에 의해 조절됨을 알 수 있었다.

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Mutagenic DNA Repair Pathways in *Aspergillus nidulans*: Effects of *uvsJ*, a *rad6* Homolog, on Survival, Cell Growth and Mutagenesis

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RAD6 protein is indispensable to generate mutations in yeast. However, the function of RAD6 is mostly unknown except its ubiquitin conjugating (UBC) activity. In *Aspergillus nidulans*, lack of mutagen-induced mutations has been observed in mutants of two different epistasis groups, UvsI and UvsC. To investigate whether the RAD6-dependent mutation pathway is also operated in *Aspergillus nidulans*, we have been cloned and characterized a *Rad6* homolog (*radB*) to find that *radB* is an allele of *uvsJ* previously assigned in UvsF group. In this study, null mutation of *uvsJ* was constructed by targeted gene replacement and the UBC enzymatic active site mutation, C88A was also generated to examine their effects on mutagenesis. Disruption of *uvsJ* caused growth retardation on an agar plate indicating its requirement on normal growth. Such a phenotype did not exhibited in *uvsJ1* mutant carrying a single point mutation at 58th amino acid histidine. We also found that *uvsJ1* was a temperature sensitive mutant showing the same level of mutagen-sensitivity to wild type at the permissive temperature 25°C but demonstrating high sensitivity at 37°C similar to *uvsJ* null mutants. In contrast to yeast *rad6* mutants, *uvsJ* null as well as *uvsJ1* mutants exhibited increased UV-induced mutation frequencies in a system detecting selenate resistant forward mutations which selects mainly the defects in the sulphate permease (*sB*) gene. Forced over-expression of UVSJ-[C88A] protein in wild type resulted in the change of colony morphology, indicating dominant-negative effects of the mutant protein on cell growth. [This work was supported by KOSEF (98-0501-005-1)]

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A Putative Timeless (TIM) Homolog of *Aspergillus nidulans* Partially Complements the MMS-sensitivity of *uvsH* Mutants

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