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Cloning of the Kinesin-Associated Proteins in *Schizosaccharomyces pombe* by Yeast Two Hybrid System

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Kinesins have been cloned in many organisms and they have major roles in movement of chromosomes and cell organells and segregation of spindle poles. We have cloned kinesin genes which were named as krp1 gene, using PCR technique in Schizosaccharomyces pombe. The *S. pombe* krp1 kinesin motor doamin was highly conserved and a member of kinesin heavy chain (KHC) superfamily. krp1 gene was not essential because cells containing a krp1 null allele were viable. Overexpression of krp1 results in the inhibition of cytokinesis; cell become elongated and branched and form aberrant septa. To examine the function of krp1 in detail, we used the yeast two hybrid system to clone the protein that interacts with the krp1. The screening identified novel proteins, designed kap1, kap2 and kap3, which exhibited a strong structural homology with myosin family.

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ArsA as a Bacterial Cell Cycle Inhibitor

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ArsA, gene product of *ars*A, is P-type ATPase and its amino acid sequence is similiar with MinD, which is a membrane-bound ATPase required for correct placement of the *Escherichia coli* division site and inhibits bacterial cell cycle when over-expressed. Our previous study revealed that the cells elongated when *ars* operon in plasmid was over-expressed, but the mutants, inserted with Tn5::*pho*A at *ars*A, did not. So we deduced its function as a bacterial cell cycle inhibitor. In this study, we amplified *ars*A from *ars* operon, cloned into pUC18 named pAE300, to construct P_{lac}::*ars*A system. And the same step was performed to the frame-shift mutant *ars*A. Then DH5a containing *ars*A was induced with IPTG, and the cell elongated. But mutant *ars*A carrying DH5a did not. *E. coli* wild type MG1655 transformed with pDB164 (P_{lac}::*min*D) showed filamentous forms and so did pAE300 (P_{lac}::*ars*A). This results demonstrate that ArsA is a bacterial cell cycle inhibitor and may complement MinD.

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Cloning and Sequencing of the cDNA Encoding Putative Mitogen-Activated Protein Kinase from a Phytopathogenic Fungus *Mycosphaerella melonia*

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MAP kinase 신호전달계는 이스트와 같은 미생물로부터 사람과 같은 고등생명체에 이르기까지 다양한 종류의 진핵세포생물에서 잘 보존되어 있으며 이들은 세포의 성장, 분열, 죽음 등 다양한 생리현상을 조절하는 것으로 알려져 있다. 그러나 식물 병원성 곰팡이에서는 MAP kinase에 대한 연구가 많이 되어있지 않다. 수박덩굴 마름병을 유발하는 *Mycosphaerella melonia*의 MAP kinase cDNA를 클로닝하기 위해 nitrogen starvation 시킨 후 total RNA를 추출 하였고 mRNA만을 정제하여 RT-PCR을 수행하였다. PCR clonging은

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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