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Cellulase gene cloning and characterization of the hyphal cellulolytic enzyme from a plant growth promoting rhizobacterium, *Bacillus licheniformis* K11

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The cellulase gene of *Bacillus licheniformis* K11 was cloned in pUC19 using PCR employing heterologous primers designed from *B. subtilis*. The 1.6kb PCR fragment contained the full sequence of the cellulase gene, denoted *cel*W, and the 1,497bp gene of *B. licheniformis* K11 cellulase predicted a 499 amino acid protein. Similarity search in protein data base revealed that the cellulase from *B. licheniformis* was more than 96% identical in amino acid sequence to those of various *Bacillus* spp. The protein from *B. licheniformis* K11 overproduced in *E. coli* DH5α, by the lac promoter on the vector, had apparent molecular weight of 55kDa upon native CMC-SDS-PAGE analysis. The enzyme protein of *B. licheniformis* K11 cellulase not only had enzymatic activity toward carboxymethyl-cellulose(CMC) but also was able to degrade insoluble cellulose, such as Avicel and filter paper. Biochemical analysis showed that the enzyme had a maximum activity at 60℃ and pH 6.0. And the enzyme activity was activated by $CoCl_2$ or $MnSO_4$. However its activity was inhibited by $FeCl_3$ or $HgCl_2$. Also the enzyme activity was activated by SDS or sodium azide.

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Transcriptional silencing of CKII catalytic subunits through DNA hypermethylation is associated with cellular senescence

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Protein kinase CKII (formerly known as casein kinase II) is a ubiquitous and highly conserved protein serine/threonine kinase, which is found in all eukaryotes examined and in various subcellular compartments. The holoenzyme of CKII is a heterotetramer, composed of two catalytic (α and/ or α′) and two regulatory (β) subunits. CKII plays a critical role in cell growth and proliferation. The present study demonstrates that CKII activity apparently decreases during both replicative and H_2O_2-induced senescence in human diploid fibroblast IMR-90 cells. The mRNA and protein levels of CKIIα and CKIIα′ decrease significantly during replicative and H_2O_2-induced senescence. Knockdown of CKIIα or α′ in IMR-90 cells by RNA interference dramatically induced the senescent phenotype. The treatment of senescent IMR-90 cells with 5-aza-2′-deoxycytidine induced CKIIα and CKIIα′ expression, indicating that DNA methylation was involved in the silencing of CKIIα and α′ genes in senescent cells. Taken together, these results suggest that the down-regulation of CKII catalytic subunits through DNA methylation is tightly associated with cellular senescence.