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Heat shock response plays a central role in cellular adaptation to stress and hostile environments. Of several heat shock proteins (hsps), DnaK (hsp70 family) and GroEL (hsp60 family) function as molecular chaperones in the folding of nascent protein chains and in the refolding of proteins after thermal damage. Expression of dnaK operon and groEL operon in S. pneumoniae and other gram positive organisms is negatively controlled by a repressor encoded by hrcA, the first gene of dnaK operon. HrcA repressor bound to CIRCE elements at the promoter site of dnaK and groEL operon. Since HrcA depends on GroEL to acquire on active conformation, GroEL seems to be specific modulator of the CIRCE regulon. Previously we demonstrated that the dnaK operon has three promoter sites and binding of HrcA to CIRCE element was stimulated by supplement of calcium. In this study, to elucidate the pathological role and expression control of dnaK operon in S. pneumoniae, nucleotide sequence of the dnaK operon was completely determined and effect of hrcA mutation on dnaK expression was examined. In the hrcA mutant, the basal and induced levels of DnaK protein and dnaK transcript were much higher than that of the wild type. When the effect of calcium on hsp expression was determined by Western blot analysis, both GroEL and HrcA proteins were decreased to the basal level by 1mM calcium but DnaK was not affected by calcium. These results suggest that calcium may play a differential roles in either promoter 1, 5' to the hrcA or promoter 3, 5' to the dnak.

[PC2-8] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

ermAM and ermK are also inducibly expressed by 16-membered-ring macrolide antibiotics.

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The *erm* family is one of the major groups of genes responsible for inducible macrolide-lincosamide-streptogramin B (MLS_B) antibiotics resistance. The 16-membered-ring macrolides have been generally considered noninducers except in the case of *Streptomyces* and the selected mutant strains. In this study, the induction specificity of *ermAM*, *ermK*, and *ermC* for MLS_B resistance was studied by *lac* reporter gene assay. The unexpected MLS_B resistance phenotypes were observed. *ermAM* was induced by the 16-membered-ring macrolides tylosin, kitasamycin, josamycin, and rokitamycin more strongly than the lincosamide clindamycin known as inducer of *ermAM*. *ermK* was only induced by the tylosin of the 16-membered-ring macrolides tested. However *ermC* was not induced by any 16-membered-ring macrolides.

[PC2-9] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Ulcerative colitis induced GAGs degrading enzymes of intestinal bacteria

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Ulcerative colitis(UC) is a non-infectious chronic intestinal inflammatory disease in humans. The mechanisms underlying their pathogenesis are not well known, although it has been long discussed.
We produced acute experimental UC models in mice by several methods (5% dextran sulfate

sodium, 5% hydrolyzed carrageenan, 2,4-dinitrochlorobenzene(DNCB)). These colitis mice all showed signs of diarrhea, occult blood, prominent regenerations of the colonic mucosa and shortening of large intestine.
Among these colitis mice, GAGs degrading enzymes of intestinal bacteria, chondroitinase and hyaluronidase, were potently induced in 5% hydrolyzed carrageenan and DNCB-induced mice models.
These hydrolyzed carrageenan and dextran sulfate sodium also exhibited the *in vitro* cytotoxicity against intestinal epithelial cell line. The compounds also induced bacterial GAGs degrading enzymes *in vitro* intestinal bacteria culture system.
Therefore, these results suggest that the supressions of a GAGs-degrading bacteria could improve a non-infectious inflammatory disease.

* Intestinal epithelial cell : IEC18 cell

[PC2-10] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

β-Glucosidases of human intestinal bacteria transforming ginsenoside Rb1 and Rb2 to compound K

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Among herbal medicines, ginseng is frequently used as a crude taken orally in Asian countries as a traditional medicine. The major components of ginseng are ginsenosides. These ginsenosides have been reported to show various biological activities including an anti-inflammatory activity and anti-tumor effects. To explain these pharmacological actions, it is thought that ginseng saponins must be metabolized by human intestinal bacteria after orally taken them.
Therefore, we investigated the metabolism of ginsenoside R_{b1} and R_{b2} by human intestinal bacteria and their metabolism-related β-glucosidase. By human intestinal microflora, ginsenoside R_{b1} and R_{b2} were metabolized these ginsenosides to compound K and 20(S)-protopanaxadiol. *Eubacterium* sp., *Streptococcus* sp. and *Bifidobacterium* sp., which hydrolyzed more potently gentiobiose than sophorose, metabolized ginsenoside R_{b1} to compound K via ginsenoside R_d rather than gypenoside XVII. However, *Fusobacterium* K-60, which hydrolyzed more potently sophorose than gentiobiose, was metabolized to compound K via gypenoside XVII. Ginsenoside R_{b2} was also metabolized to compound K via ginsenoside R_d or compound O by human intestinal microflora. *Eubacterium*sp., *Streptococcus* sp. and *Bifidobacterium* sp. metabolized ginsenoside $R_{b2}$to compound K via ginsenoside R_d rather than compound O. *Fusobacterium* K-60 metabolized ginsenoside R_{b2} to compound K via compound O.

[PC2-11] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

The Gene Cloning of a Chitinase from Cytophaga sp. HJ

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Cytophaga sp. HJ is a bacterial strain producing an extracellular chitinase, induced by chitin. The chitinase gene was cloned in Escherichia coli JM109 by using pUC18. A clone expressing chitinase activity was obtained from about 670 transformants. It had 12.7Kb plasmid DNA. When the plasmid was digested by HindIII, we found that it contained 10Kb insert DNA fragment as well