A novel penicillin G acylase (PGA)-producing bacterial strain was isolated from soil by using the *Serratia marcescens* overlay technique. The isolated strain was identified as *Leclercia adecarboxylata* based on the analyses of the biochemical characteristics (API 20E), the cellular fatty acid profile, and the 16S rDNA sequences. The gene encoding the PGA (*pac* gene) was cloned into the pHSG399 vector and the recombinant *E. coli* HB101 clones harboring the *pac* gene were isolated on agar plates containing phenylacetyl-L-leucine and penicillin G. DNA sequence analysis revealed that the cloned *pac* gene encodes a polypeptide which is the precursor of a typical periplasmic, class IIa penicillin G acylase. This was consistent with the strong homology of the entire DNA sequence with other *pac* genes from the known PGA-producing organisms including *Kluyvera citrophila* (77% identity) and *Escherichia coli* (75% identity). The predicted amino acid sequence of the *pac* gene product consists of the N-terminal signal peptide region, the α subunit of the PGA, a spacer peptide region and the β subunit of the PGA. At least one consensus CRP-binding motif was found in the vicinity of the upstream promoter region. Expression of the *pac* gene was regulated by phenylacetic acid, glucose and growth temperature in both wild-type and the recombinant strains. The enzyme was purified to near homogeneity by using ammonium sulfate precipitation, DEAE-Sepharose and Bio-Gel hydroxyapatite column chromatography. The purified enzyme appeared as two distinct polypeptides (α and β subunits) on a SDS polyacrylamide gel and the apparent molecular weights of α and β subunits were 26 and 65 kDa, respectively. The N-terminal sequence analyses of the subunits were performed in order to locate the precise processing points in the prepropeptide. Native molecular weight of the enzyme estimated by Superose 12 gel-filtration chromatography suggests that the enzyme exist as a heterodimer as in the cases of other class IIa PGA enzymes.

[PC2-4] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Cloning, Sequencing and Characterization of the Urease Gene Cluster of the *Streptococcus vestibularis*

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Streptococcus vestibularis is a urease-producing oral bacterium, frequently isolated from vestibular mucosa of human oral cavity. Ureolysis by *S. vestibularis* and other ureolytic oral bacteria is believed to be crucially involved in oral microbial ecology and oral health. Genomic library of the *S. vestibularis* ATCC49124 was constructed in an *E. coli* plasmid vector and the urease-positive transformants harboring the urease gene cluster were isolated on Christensen-urea agar plates. The minimal DNA region required for the urease activity was located on a 5.6 kb DNA fragment. DNA sequence analysis revealed the presence of partial *ureI* gene and seven complete open reading frames, corresponding to *ureA, B, C, E, F, G* and *D*, respectively. The nucleotide sequence over the entire *ure* gene cluster and the 3'-end flanking region of *S. vestibularis* was 92% identical to that of *Streptococcus salivarius*, which is another closely related oral bacterium. The predicted amino acid sequences of the structural peptides were 98-99% identical to the corresponding peptides of *S. salivarius*, and the accessory proteins were 94-99% identical each other. The recombinant *E. coli* strain containing the *S. vestibularis ure* gene cluster expressed high level of functional urease holoenzyme when grown in a medium supplemented with 0.8-1.5 mM nickel chloride. The enzyme was purified over 49-fold by using DEAE-Sepharose, Superdex 200, BioScale-Q and Mono-Q column chromatography. Specific activity of the purified enzyme was 2.019 μmol ammonia/min/mg protein and the Km was estimated to be 1.45 mM. Apparent molecular weights of the three structural protein subunits on a 10-15 % gradient SDS-polyacrylamide gel were 11.6, 14.9 and 64.2 kd, respectively.

[PC2-5] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Three alkaline proteases of Bacillus spp. JB411.

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Three alkaline proteases, designated JB-1, JB-2, and JB-3, are extracellular enzymes produced by Bacillus spp.

JB411 which was isolated korean soil. They were separated by DEAE-sepharose CL-6B gel, and further purified using ammonium sulfate precipitation, ultra membrane filtration, and Ultrogel AcA gel filtration. The optimun pH values of proteases IB-1, JB-2, and JB-3, were shown to be 9.5, 9.5 and 7.5, respectively. All three proteases were stable in the pH range of 5-11. The maximum activities for the enzymes were 60℃, 55℃, and 55℃, respectively. All three proteases were inhibited by phenylmethylsulfonylfluride(PMSF), whereas the metal chelators EDTA and EGTA did not affect enzyme activities. Enzyme activities of protease JB-1 and JB-2 were enhanced by Triton X-100 and Tween 80. Metal ions did not significantly affect protease JB-1 activity, whereas protease JB-2 was slightly inhibited by several metal ions (Co2+, Fe2+, Ni2+, Zn2+).

[PC2-6] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Biochemical analysis of a high-molecular-weight protease from Streptomyces tendae JC412

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Streptomyces tendae JC412 secreted two forms of protease(ST-1 and ST-2) when grown in OSY medium (oatmeal 1.5%, soybean meal 2%, dried yeast 1%) supplemented with glucose(0.5%) and KH2PO4(0.05%). Initial pH of the culture medium was adjusted to 10.0 with NaOH and incubated at 27℃ on a rotary shaking incubator (180rpm). High- molecular-weight protease ST-1 was heat labile, whereas low molecular protease ST-2(22,000 Da) was reported to be heat stable. Protease ST-1 was purified through Ultrogel AcA 54 and DEAE-sepharose CL-6B column chromatography. Protease ST-1 was practically stable in the pH range of 5-9. The optimum temperature for the activity of protease ST-1 was 55℃, and about 60% of the original protease ST-1 activity remained after being treated at 45 ℃ for 30min. Protease ST-1 was strongly inhibited by the metal chelators EDTA and EGTA, whereas phenylmethylsulfonylfluride (PMSF), a serine protease inhibitor, did not showed any significant effect on the enzyme activities. Protease ST-1 was unstable against H2O2 and SDS, but stable against acetone, urea, and Tween 80. Cu2+ and Ni2+ inhibited enzyme actvity of protease TA-1.

[PC2-7] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Purification and Characterization of β-Xylosidase from *Bifidobacterium breve* K-110

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Kakkalide from *Puerariae Flos* expresses pharmacological actions after biotransformation to irisolidone by intestinal bacteria. *B. breve* K-110 was isolated as a bacterium metabolizing kakkalide. Therefore, we purified kakkalide-metabolizing β-Xylosidase from *B. breve* K-110.
β-Xylosidase from *B. breve* K-110 (isolated from Korean intestinal microflora) was induced by kakkalide. We used defined medium containing 1mM kakkalide for the cultivation of *B. breve* K-110. From the precipitate of 10 L cultured bacteria, β-xylosidase was purified by 70% ammonium sulfate fraction and chromatography on QAE-Cellulose, Butyltoyopearl, Hydroxyapatite, High-trap Q-sepharose, Sephacryl S300 column. Specific activity of β-xylosidase was 103.25 μmole/min/mg. Molecular weight of β-xylosidase was 47,500 daltons by SDS-PAGE and gel filtration. The enzyme was inhibited by MnCl2 and activated by BaCl2. And its optimal pH was 5.0-5.5. The purified enzyme biotransformed kakkalide to irisolidone glycoside.

[PC2-8] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]