Alkaline protease of Actinomycetes CS0703 : Isolation, production and characterization

Kim JoonHo, Yoo JinCheol[O]

Department of Pharmacy, College of Pharmacy, Chosun University, Kwangju 501-759, Korea

Actinomycetes CS0703 has been isolated in soil sample from location in the Jeju province, Korea, and produces alkaline extracellular proteases. To maximize protease production, initial pH of the culture medium was adjusted to 12.0 with NaOH and incubated at 48℃ on a rotary shaking incubator(180rpm). Actinomycetes CS0703 produced high level of protease at late exponential phase when grown in OSYM medium (oatmeal 2.0%, soybean meal 1%, dried yeast 1%, mannitol 1%). One major protease(AA-1) was purified through ammonium sulfate precipitation, Ultrogel AcA 54, and DEAE-sepharose CL-6B column chromatography. Protease AA-1 was practically stable in the pH range of 4-10. About 66% of the original protease AA-1 activity remained after being treated at pH 11.5 for 1 hour. The optimum temperature and pH for the activity of protease AA-1 were 65℃ and 10.5, respectively. About 48% of the original protease AA-1 activity remained after being treated at 60℃ for 30min. Protease AA-1 was inhibited by phenylmethylsulfonyl- fluride(PMSF), a serine protease inhibitor. Protease AA-1 was stable against EDTA, EGTA, H2O2, EtOH, and MeOH. Triton X-100 and Tween 80 enhanced the enzyme activity, whereas metal ions did not significantly affect protease activity.

[PC2-2] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Isolation, production, purification and biochemical properties of thermostable protease produced by actinomycetes CS0707 isolated from Korean soil.

Nam DooHyun1, Choi CheolHee2, Kee KeunHong3, Kim SiWouk4, Han JiMan5, Kim JoonHo[O]5, Lee HyoJung5, Yoo JinCheol5

5Department of Pharmacy, College of Pharmacy, Chosun University, Kwangju 501-759

1Department of Pharmacy, Yeungnam University, Kyongsan 712-749, Korea
2Department of Pharmacology and 3Department of Patholgy, College of Medicine, Chosun University, Kwangju 501-759, Korea
4Department of Environmental Engineering, Chosun University, Kwangju 501-759, Korea
5Department of Pharmacy, College of Pharmacy, Chosun University, Kwangju 501-759.

Actinomycetes CS0707 has been isolated in soil sample from location in the Jeju province, Korea, and produces thermostable extracellular proteases. Actinomycetes CS0703 showed the highest protease activity at late exponential phase when grown in OSYM medium (oatmeal 2.0%, soybean meal 1%, dried yeast 1%, mannitol 1%) at 48℃. Three forms of protease(TA-1, TA-2, and TA-3) were fractionated by Ultrogel AcA 54 column chromatography, and further purified through ammonium sulfate fractionation, ultramembrane filtration, and DEAE-sepharose CL-6B column chromatography. The optimun pH values of proteases TA-1, TA-2, and TA-3, were shown to be 7.5, 6.5 and 10.0, respectively. Protease TA-1, TA-2, and TA-3 were stable in the pH range of 6-11.5, 4-9, and 5-11, respectively. The optimum temperature for the activities of protease TA-1, TA-2 and TA-3 were 55℃, 65℃, and 65 ℃, respectively. Above 50% of the original protease activities(TA-1, TA-2, and TA-3) remained after being treated at 60℃ for 30min. Protease TA-1 was inhibited by the metal chelators EDTA and EGTA, whereas phenylmethylsulfonylfluride(PMSF) did not affect enzyme activity of TA-1. ies. Protease TA-2 and TA-3 were strongly inhibited by phenylmethylsulfonyl- fluride(PMSF), a serine protease inhibitor. EDTA and EGTA did not inhibit protease TA-2 and TA-3.

[PC2-3] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Cloning, Sequencing and Characterization of the Novel Penicillin G Acylase Gene from the Soil-isolated *Leclercia adecarboxylata*

Sang O. Jun[O], Ho S. Lim, Geun Y. Kim, Eung S. Lee, Mann H. Lee

Catholic Univ. of Daegu, College of Pharmacy, Hayang, Kyongsan, Kyongbook, 712-702 Republic of Korea

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*

Geun Y. Kim°, Mann H. Lee

Catholic Univ. of Daegu, College of Pharmacy, Hayang, Kyongsan, Kyongbook, 712-702 Republic of Korea

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.

Lee OhHyoung1, Seong ChiNam2, Park GiDuk3, Kim AnNa3, Han JiMan3, Lee HyoJung3, Kim SulHee°3, Yoo JinCheol3

Department of Pharmacy, College of Pharmacy, Chosun University, Kwangju 501-759, Korea

1Department of Biology, Mokpo National University, Chon Nam, Korea 534-729
2Department of Biology, Sunchon National University, Chon Nam, 540-742, Korea
3Department of Pharmacy, College of Pharmacy, Chosun University, Kwangju 501-759, Korea

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