

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-Sephadex 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-Sephadex, 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 K_m 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.