

JB411 which was isolated from 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 optimum pH values of proteases JB-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°C, 55°C, and 55°C, respectively. All three proteases were inhibited by phenylmethylsulfonyl fluoride (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°C 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°C, and about 60% of the original protease ST-1 activity remained after being treated at 45°C for 30min. Protease ST-1 was strongly inhibited by the metal chelators EDTA and EGTA, whereas phenylmethylsulfonyl fluoride (PMSF), a serine protease inhibitor, did not show 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 activity 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 μ mole/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]