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 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 phenylmethylsulfonylfluoride (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

Seong ChiNam1, Nam DooHyun Nam2, Kim SungJun3, Cho SeungSik4, Han JiMan4, Kim JoonHo4. Lee HyoJung5, Yoo JinCheol Yoo4

Department of Pharmacy, College of Pharmacy, Chosun University, Kwangju 501–759.

1Department of Biology, Sunchon National University, Chon Nam, 540–742, Korea
2Department of Pharmacy, Yeungnam University, Kyongsan 712–749, Korea
3Department of Genetic Engineering, Chosun University, Kwangju 501–759, Korea
4Department of Pharmacy, College of Pharmacy, Chosun University, Kwangju 501–759.

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 stable, 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 phenylmethylsulfonylfluoride (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 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

Shin HoYoung0, Han YeoOk, Han MyungJoo, Lee JangYeon, Lee JiHyun, Kim DongHyun

College of Pharmacy, KyungHee University

Kakkalide from *Pueraaria 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, Hydroxypatite, 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 ]