Alkaline protease of Actinomycetes CS0703 : Isolation, production and characterization

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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.

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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*

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