Purification and characterization of an extracellular cold-active serine protease from the psychrophilic bacterium *Colwellia* sp. NJ341

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**Abstract**

*Colwellia* sp. NJ341, isolated from Antarctic sea ice, secreted a cold-active serine protease. The purified protease had an apparent Mr of 60 kDa by SDS-PAGE and MALDI-TOF MS. It was active from pH 5–12 with maximum activity at 35°C (assayed over 10 min). Activity at 0°C was nearly 30% of the maximum activity. It was completely inhibited by phenylmethylsulfonyl fluoride.

**Introduction**

Cold-active or cold-adapted enzymes are produced by organisms existing in permanently cold habitats. Cold-active metalloproteases and cellulases have been used as cleaning detergents, in leather processing, food processing and molecular biology (Cavicchioli et al. 2002). Bacteria living within Antarctic sea ice have a high potential for biotechnological applications (Russell et al. 1997) as they produce a variety of cold-active enzymes (Nichols et al. 1999). However, we believe that this is the first report of the purification and characterization of an extracellular cold-active serine protease from a psychrophilic bacterium, *Colwellia* sp. NJ341.

**Materials and methods**

**Bacteria and their cultivation**

Two hundred sixty strains were isolated from the sea ice in Antarctica (68°30′E, 65°00′S) during 2001–2002. Strains were conserved in the Key Laboratory of Marine Bio-active Substances, Qingdao, China. Strains were inoculated in the sea water medium (peptone 0.5% and yeast extract 0.1%, pH 7.5) at 8°C with shaking at 100 rpm.

**Assay of protease activity**

Protease activity was determined by a modified method of Folin & Ciocalteau (1927). Briefly, 50 μl purified protease was added to the reaction mixture containing 2% (w/v) casein in 50 μl 50 mM Tris/HCl (pH 8). The mixture was incubated at 35°C for 10 min, the reaction stopped by adding 100 μl 10% (w/v) trichloroacetic acid, and centrifuged at 9000×g for 15 min. The protein remaining in the supernatant was determined by the Folin-pheno reagent. One unit of protease activity was defined as the amount of enzyme that liberated 1 μg tyrosine per min. A blank was run in the same manner except the enzyme was added after the addition of 10% (w/v) TCA. All experiments were done in duplicate.
Protein determination and zymogram

Protein was assayed according to Lowry using bovine serum albumin (BSA) as a standard. A zymogram was performed as described by Liu et al. (1997).

Results and discussion

Characterization of Colwellia sp. NJ341

Protease activity of the cultures was screened from 260 strains of Antarctic bacteria taken from sea ice, and strain NJ341 showed the highest protease activity (126 U ml\(^{-1}\)). Strain NJ341 was identified as the genus *Colwellia* from its biochemical characteristics and 16S rDNA sequence. Phylogenetic tree analysis by neighbor-joining method also exhibited that strain NJ341 was more closely related to the genus *Colwellia* (levels of similarity, 89.8–94.8\%), and its closest relative was *C. piezophila*. It was named *Colwellia* sp. NJ341.

Purification of an extracellular cold-active protease

The purification of the protease was summarized in Table 1. A purification of approx. 31-fold with a yield of 9% was achieved. The SDS-PAGE analysis of the purified enzyme revealed a single band and the concentrated protein with protease activity was reexamined by zymogram and exhibited a single active protein band (Figure 1). MALDI-TOF MS of the purified protease gave a single peak with an approx. Mr of 60 kDa (Figure 2a). With the peptide masses obtained from MALDI-TOF MS of tryptic peptides (Figure 2b), extensive searches from protein databases in NCBI did not yield any other protease sequences to this protease.

Effect of pH and temperature on the protease activity and its stability

The purified protease from *C*. sp. NJ341 exhibited the maximum activity at pH 8–9 (Figure 3a). The maximum activity was about 35°C and its protease activity under 0°C exhibited nearly 30%.