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Improving production of extracellular proteases by random mutagenesis and biochemical characterization of a serine protease in *Bacillus subtilis* S1-4

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ABSTRACT. The feather is a valuable by-product with a huge annual yield produced by the poultry industry. Degradation of feathers by microorganisms is a prerequisite to utilize this insoluble protein resource. To improve the degrading efficiency of feathers, mutagenesis of the bacterium *Bacillus subtilis* S1-4 was performed. By combining ultraviolet irradiation and N-methyl-N'-nitro-N-nitrosoguanidine treatment for mutagenesis, a high protease-producing mutant (UMU4) of *B. subtilis* S1-4 was selected, which exhibited 2.5-fold higher extracellular caseinolytic activity than did the wild-type strain. UMU4 degraded chicken feathers more efficiently, particularly for the release of soluble proteins from the feathers, compared to the wild-type strain. Furthermore, an extracellular protease with a molecular weight of 45 kDa, as determined by SDS-PAGE, was purified from UMU4. Biochemical characterization indicated that the caseinolytic activity of the protease was largely inhibited by phenylmethanesulfonyl fluoride, suggesting that the purified enzyme is

a serine protease. This protease was highly active over a wide range of pHs (6.0 to 12.0) and temperatures (50° to 75°C) with an optimal pH and temperature of 8.0 and 65°C, respectively. The purified enzyme exhibited good thermostability with a 72.2 min half-life of thermal denaturation at 60°C. In addition, this protease was not sensitive to heavy metal ions, surfactants, or oxidative reagents. In conclusion, strain improvement for protease production can serve as an alternative strategy to promote feather degradation. The UMU4 mutant of *B. subtilis* and its serine protease could be potentially used in various industries.

Key words: *Bacillus subtilis*; Mutagenesis; Strain improvement; Purification; Serine protease

