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Bacillus pseudofirmus AL-89

A source for industrial relevant proteases

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The following study describes the production of *Bacillus pseudofirmus* strain AL-89, purification and characterization regarding an alkaline protease from *Bacillus pseudofirmus* strain AL-89. The effect of nutrient medium on growth and enzyme production were investigated with different kinds of peptone (casein peptone and meat peptone) and different glucose concentrations (2 g/l and 5 g/l) at pH 10 and 37°C in a controlled batch reactor.

It was shown that *Bacillus pseudofirmus* strain AL-89 produces 20% more biomass in presence of meat peptone than in casein peptone cultures. Highest specific enzyme activity of $1.97 \cdot 10^5$ unit/g DM for azo-casein hydrolysis was measured in a culture with meat peptone and 5 g/l glucose.

The enzyme preparation, purified by ammonium sulphate precipitation, ion exchange- and size exclusion chromatography, showed a pH profile with maximum activity at 55°C and pH 8-8.5 and in addition a shoulder between pH 10 and 11. This indicated either that at least two enzymes were present in the preparation or one enzyme with increased activity at different pH values. Previous investigation revealed that *Bacillus pseudofirmus* strain AL-89 produces an alkaline protease called AL-89 with maximum activity at 60°C and pH 10.5-11. The same enzyme was identified by mass spectrometry investigation and was probably responsible for the shoulder between pH 10 and 11. The adding of phenylmethylsulfonyl fluoride as a specific Ser-protease inhibitor revealed to a decrease in activity. That leads to the conclusion the enzymes in the preparation may belong to the clan of Ser-proteases.

Furthermore, it could be shown that *Bacillus pseudofirmus* strain AL-89 produces more than one proteolytic enzyme simultaneously with significant activity.