ABSTRACT

*B. megaterium* SN1, *B. thuringenesis* SN2, *B. pumilis* SN3 isolated from soil of Ghazipur poultry waste site, a feather dumping site. They produced extracellularly keratinolytic and caesinolytic enzyme in feather meal media 2.

All bacterial isolates showed antibacterial activity, presence of caesinolytic and Milk clotting activity in crude and ammonium sulphate fractions. Highest ratio (520.84) of milk clotting activity to caesinolytic activity was seen of these strains. *B. megaterium* SN1 and *B. thuringenesis* SN2 produced extracellular caesinolytic and keratinolytic enzyme showing optimum pH 3-5 at 30°C, 160 rpm after 72 hrs and 96hrs which remained active at 60°C - 70°C respectively. Enzyme from *B. megaterium* SN1 was purified by ammonium sulphate precipitation, 25 Q sephr ose chromatography. Casein zymography studies showed that enzyme is 30 kDa. Mn$^{2+}$ strongly activated caesinolytic and keratinolytic enzyme activity whereas Hg$^{2+}$ inhibited caesinolytic and keratinolytic enzyme activity.

Enzyme from *B. thuringenesis* SN2 was purified by ammonium sulphate precipitation. Casein zymography studies showed that enzyme has subunits of 120 kDa, 80 kDa, 60 kDa and 40 kDa. Mn$^{2+}$ and Ba$^{2+}$ strongly activated caesinolytic and keratinolytic enzyme activity by 3.7, 1.9 fold whereas Ba$^{2+}$ and Fe$^{2+}$ inhibited caesinolytic and keratinolytic enzyme activity.

RSM and the Resilient back propagation, neural network was used to predict the best combination of the crucial media components for production of these enzymes. We report that there is 2.5 fold increase over the previous setups using the following predicted combination NaCl, 0.2%; Yeast extract, 1%; Feather, 15%. Trained network is a better option to predict new data points.