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Keywords:

Results

. e gene for chitin deacetylase was cloned from *Bacillus cereus* into pET22b vector (Figure 1). . e resulting construct with six His tag was subsequently expressed in *E. coli* pLysS cells. . erea er the cells were induced with IPTG at a concentration 1mm and cultivated at 18 C for 20 hours. As it is known that *E. coli* has a leaky expression in all its compartments, di. erent compartments were tested for the expression of the protein. A SDS-PAGE was run and subsequently western blotting was performed (Figure 2). As the extracellular fraction comprised a sizeable amount of the expressed CDA, this fraction was further puri ed using Ni-NTA matrix with the speci cations as per the manufacture (Qiagen, Germany) (Figure 3). . e CDA upon puri cation revealed two bands, the higher band corresponding to around 32 kDa and lower to 30 kDa. . e western blotting in gure 2 also bore two bands wherein the higher band may correspond to the protein with the signal peptide and the lower without it which could be a result of partial digestion of the signal peptide by the proteases. A er purifying the CDA, the pH optima were determined using di. erent sets of bu. ers with overlapping bu. ering range (Figure 4). . e pH range of 3-10 was interrogated with an overlap at pH 6, 7 and 8 to study the e. ect of the pH and the bu. er. It is known that co-factors play an important role in the enzyme activity of chitin deacetylase. In lieu of the same, co-factors in the form of their respective chloride salts were included in the assay mixture (Figure 5). It can be noted from gure 5 that the

gure pH optima of BcPdA e pH range from to was tak n with an o rlap

enzyme activity was the highest in presence of CO as the co-factor. e concentration of CO was later tested with a range from 0.5 mm to 10 mm (Figure 6). It can be observed that a concentration of 2 mm CO yielded the best activity of the enzyme in terms of its deacetylation activity. At the end the e ect of temperature on the activity of CDA was checked with reference to the activity which was checked on the amount of acetate released (Figure 7) with the Boehringer kit and it was found that the enzyme worked well at a temperature of the substrate was incubated for 20 h with the substrate at 37 C in Bis-Tris bu er 50 mM, pH 7.0. Hence the chitin deacetylase was characterised in terms of its pH, temperature optima and the type of co-factor required for its activation.

Discussion