

Probing the determinants of optimal pH-activity profiles of Family 1.4 Bacillus lipases

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The genes encoding extracellular alkaline lipases from *Bacillus licheniformis* and *B. pumilus* have been cloned and sequenced and have been classified as members of Family 1.4 of bacterial lipase proteins. Although the mature lipase proteins from *B. licheniformis* and *B. pumilus* share an amino acid identity of more than 95 percent they exhibit different biochemical properties. The lipase from *B. licheniformis* has a broad pH optimum (between 8.5 to 11.5) while the lipase from *B. pumilus* shows a sharp peak of optimum pH profile (pH 9.5) with pNP-palmitate as the substrate. The PCR was used to mutate Thr28 to Ala28 in the mature *B. pumilus* lipase. The mutant gene was subcloned using Nde1 and Xho1 sites into pET20b(+) such that the expressed lipase protein contained at its C-terminal a 6X His tag that facilitated protein purification by Ni²⁺-NTA affinity chromatography. The purified recombinant lipase protein revealed pH activity profile similar to that of the recombinant lipase from *B. licheniformis*, suggesting that Thr28 is the determinant of a narrow pH-activity profile in *B. pumilus* lipase. We used three-dimensional structure modelling with the mature lipase from *B. subtilis* as the template to elucidate structural and functional roles of Thr28 within Family IV Bacillus lipases.