

Enhanced Production of Acid Phosphatase in *Bacillus subtilis*: From Heterologous Expression to Optimized Fermentation Strategy

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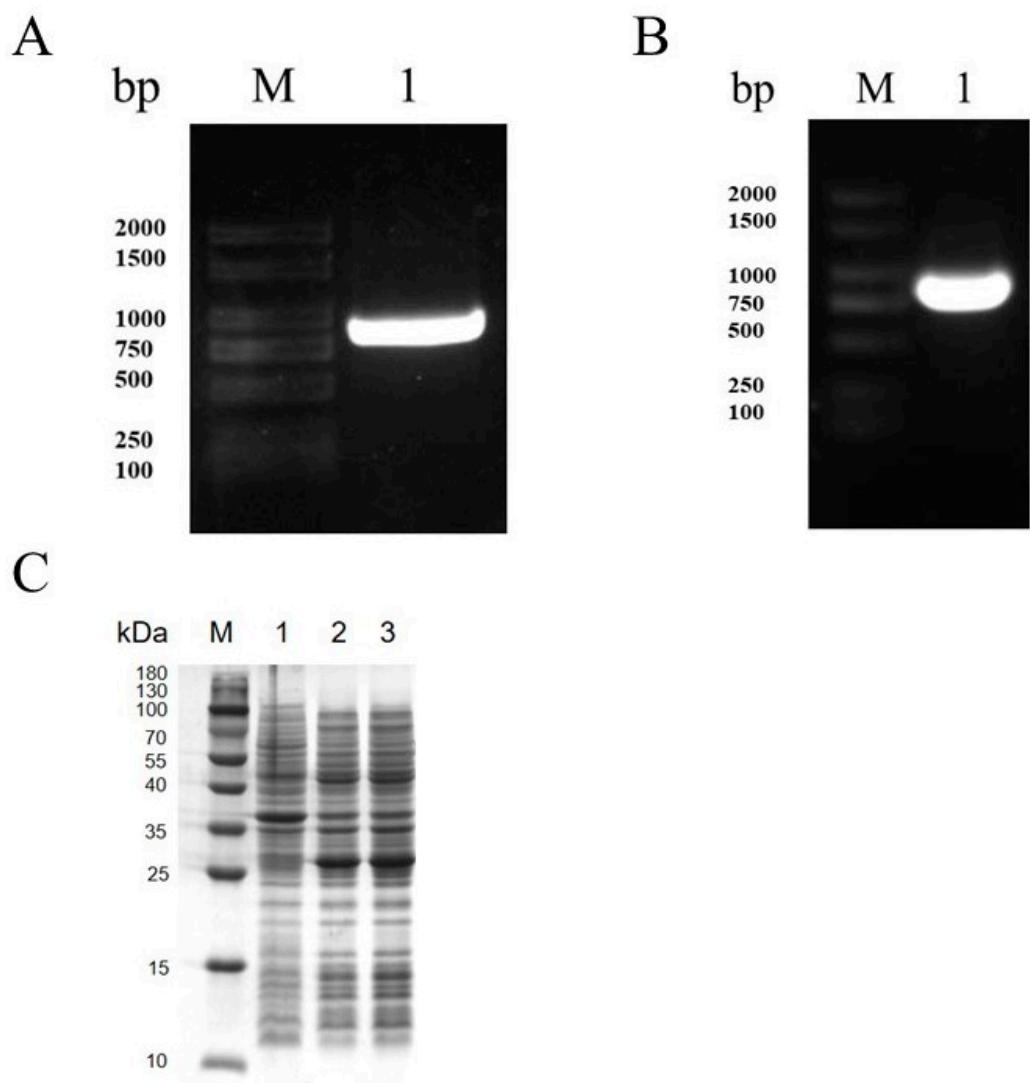


Figure S1 Recombinant *Bacillus subtilis* expressing acid phosphatase. (A) Amplification product of ACPase gene (M: Marker DL2000; Lane 1: ACPase gene). (B) Colony PCR verification (M: Marker DL2000; Lane 1: *Acp* gene). (C) SDS-PAGE analysis of acid phosphatase (M: Marker; Lane 1: whole proteins in *B. subtilis* 168/pMA5, Lane 2 and Lane 3: whole proteins in *B. subtilis* 168/pMA5-*Acp*).

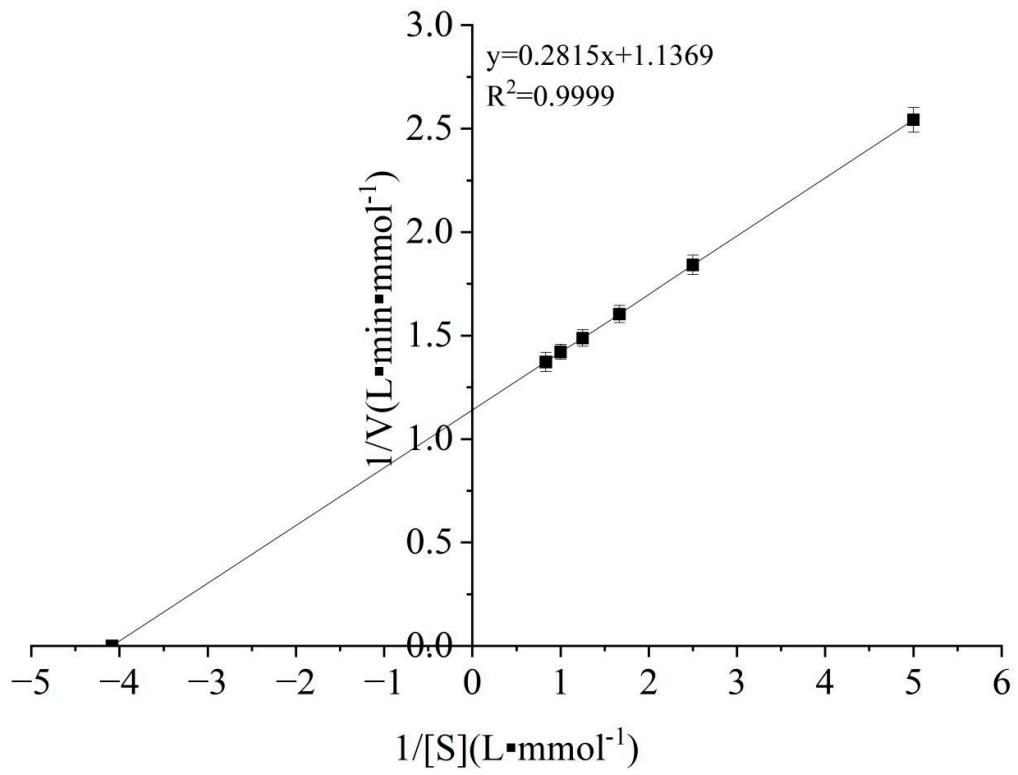


Figure S2 Determination of K_m of ACPase by Lineweaver-Burk plot.

Table S1 The purification of enzyme ACPase

| Purification steps | Total protein (mg) | Total activity (U) | Enzyme activity (U/mL) | specific activity (U/mg) | Purification factor | Recovery rate (%) |
|------------------------|--------------------|--------------------|------------------------|--------------------------|---------------------|-------------------|
| Crude enzyme solution | | | | | | |
| Ni column purification | 6.68 | 866.2 | 86.62 | 129.6 | 2.17 | 45.7 |