

**Supplementary 1.** Phylogenetic analysis of 16S rRNA gene sequence similarities of *Enterobacter* sp. 3TP2A based on the BLAST result using the neighbor-joining method. Scale bar represents 0.1 substitutions per nucleotide position. The organisms and GeneBank accession numbers of analyzed sequences are given in parenthesis.



**Supplementary 2**. Molecular weight estimation by gel filtration chromatography a) Elution profiles of gel filtration chromatography, b) Calibration curve for molecular weight determination using gel filtration chromatography.  Standard proteins; (1) β-galactosidase (MW: 116 kDa), (2) α-amylase (MW: 55 kDa), (3) carbonic anhydrase (MW: 29 kDa), (β-Gal) Purified β-galactosidase from *Enterobacter* sp. 3TP2A



**Supplementary 3a.** Effect of temperature on β-galactosidase activity in *Enterobacter* sp. 3TP2A



**Supplementary 3b.** Effect of pH on β-galactosidase activity in *Enterobacter* sp. 3TP2A



**Supplementary 4a.** Effect of thermal stability on purified β-galactosidase activity from *Enterobacter* sp.3TP2A



**Supplementary 4b.** Effect of pH stability on purified β-galactosidase activity from *Enterobacter* sp.3TP2A

**Supplementary 5.** Effect of metal ions on the activity of purified β-galactosidase from *E. cloacae*

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| --- | --- |
| **Chemicals**  | **Percent activity retained (%)** |
| **1 mM** | **2mM** | **5 mM** | **10 mM** | **20 mM** |
| Ca2+ | 94±2.3 | 95±1.5 | 100±1.5 | 105±1.0 | 84±2.1 |
| Cu2+ | 4.1±0.1 | 0 | 0 | 0 | 0 |
| Mg2+ | 117±1.5 | 125±2.3 | 120±0.3 | 120±1.5 | 147±2.3 |
| Zn2+ | 68±1.8 | 73±0.1 | 92±2.9 | 103±2.7 | ND |
| EDTA | 32±0.9 | 29±0.4 | 27±2.4 | 25±0.3 | 24±0.8 |

**ND:** Not determined

**Supplementary 6.** Effect of inhibitors on the activity of purified β-galactosidase

|  |  |
| --- | --- |
| **Chemicals**  |  **Percent activity retained (%)**  |
| **1mM**  | **2mM**  | **4mM**  | **8mM**  |
| N-ethylmaleimide  | 0  |  0  |  0  |  0  |
| DTT  | 100±1.5  | 102±2.1  |  97±0.3  | 108±1.9  |
| β-Mercaptoethanol  | 102±1.2  | 99±1.4  |  ND  | 114±1.5  |
| Iodoacetamide | 99±3.02  | 87±4.3  |  94±1.4  |  93±1.7  |
|  | **0.2 mM**  | **0.4 mM**  | **1 mM**  | **2 mM**  |
| PCMB  | 13.7±0.4  | 13.9±0.5  | 13.08±0.8  | 13.3±0.2  |

**Supplementary 7.** Linewever-Burk plot of the enzyme using various *o*NPG concentrations

