Supplementary Materials

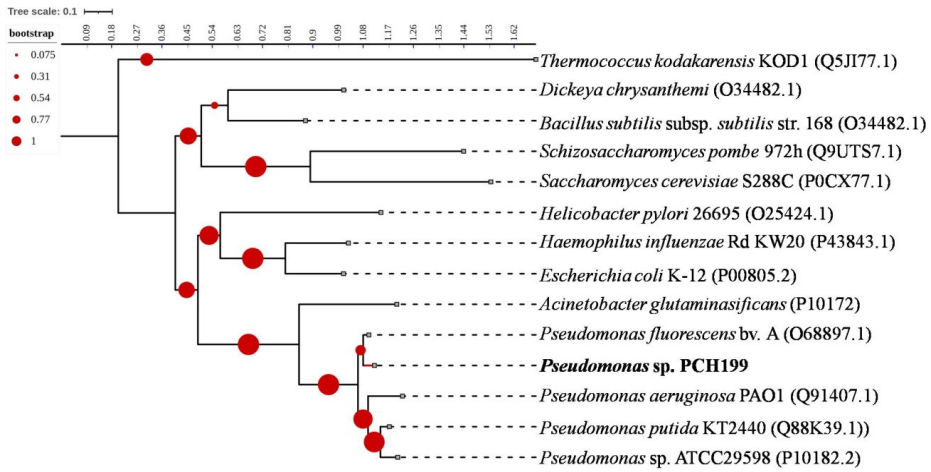

Figure S1. Phylogenetic analysis of *Pg*-ASNase II proteins of *Pseudomonas* sp. PCH199. Based on an amino acid sequence alignment of L-ASNases proteins, a rooted phylogenetic tree was constructed with the NGPhylogeny.fr and annotated using the iTOL program. The scale bar corresponds to 0.1 amino acid change per site. The UniProt ID of all sequences is indicated in parentheses.

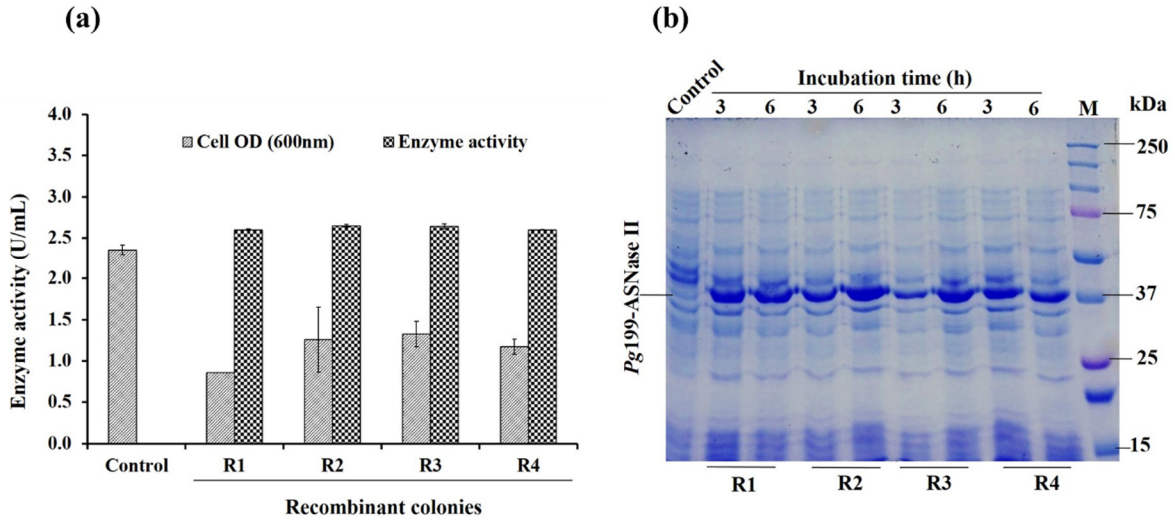

Figure S2. Expression of His-tagged *Pg*-ASNase II in LB media. **(a)** *Pg*-ASNase II (U/mL) activity and cell OD_{600} of recombinant *E. coli* BL21(DE3) after 6 h of induction with 0.5 mM IPTG. **(b)** Expression of recombinant colonies at 3 and 6 h of incubation at 37 °C in 12% SDS-PAGE.

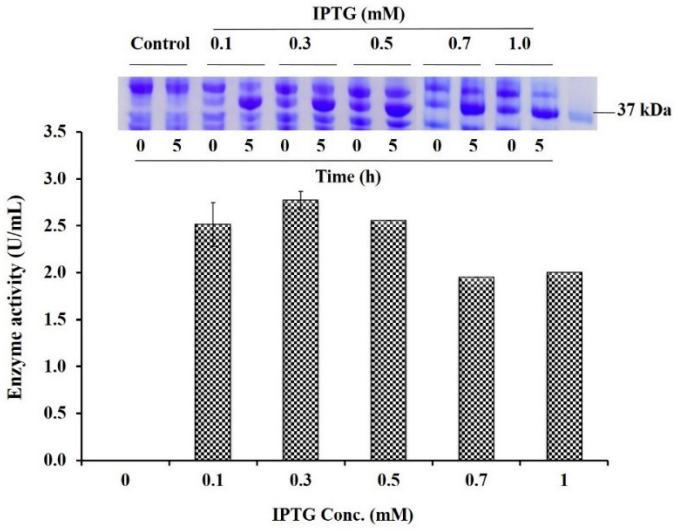

Figure S3. Expression of His-tagged *Pg*-ASNase II in *E. coli* BL21(DE3) at different IPTG concentrations. Enzyme activity (U/mL) of recombinant *E. coli* BL21(DE3) in LB medium using 0.1 to 1.0 mM IPTG concentration and 12% SDS-PAGE analysis profiles of *Pg*-ASNase II expressed at different IPTG concentrations for 0.0 and 5.0 h after induction.

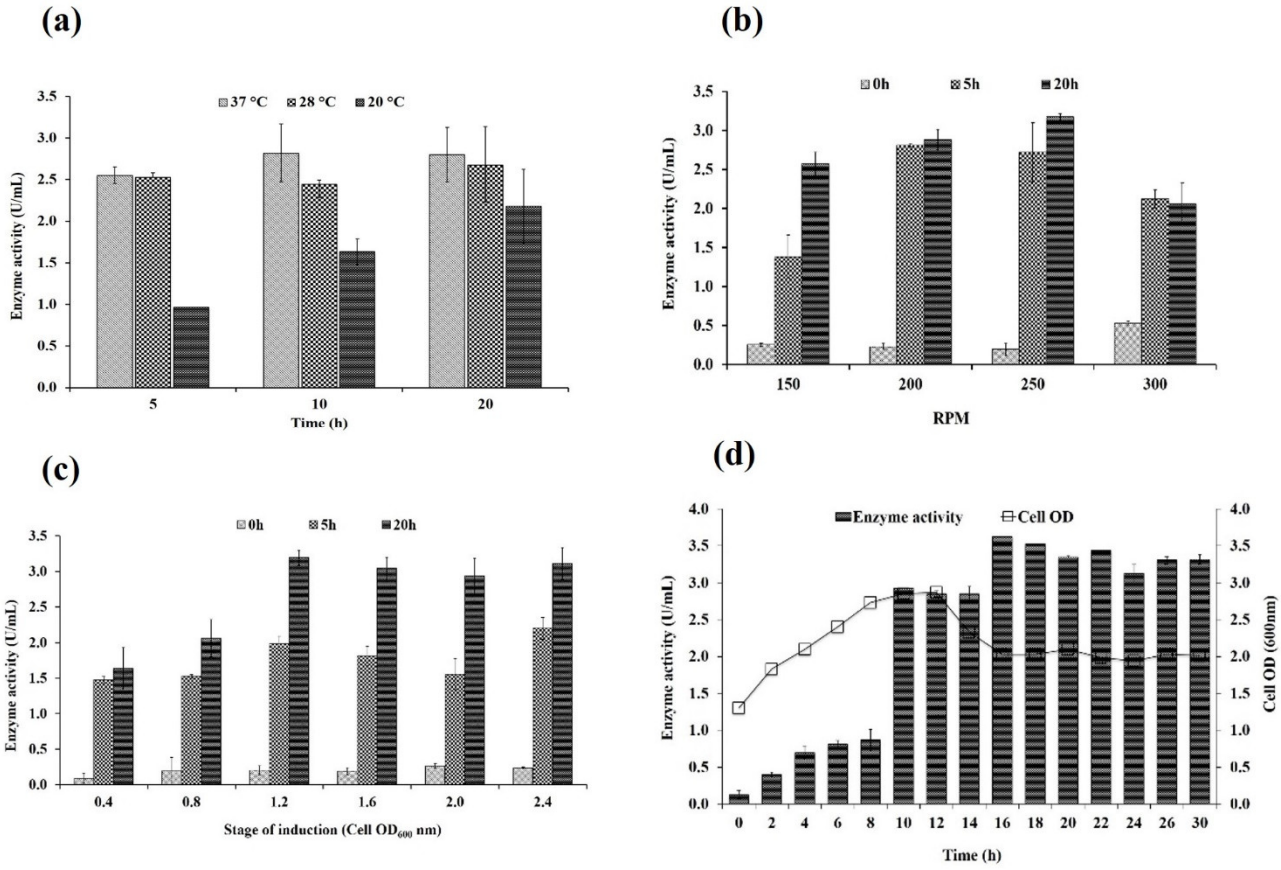

Figure S4. Production profile of His-tag *Pg*-ASNase II by recombinant *E. coli* grown in LB medium. **(a)** Effect of different levels of incubation temperature on the production of *Pg*-ASNase II activity after 5, 10, and 20 h of incubation at 20, 28, and 37 °C. **(b)** Effect of agitation on the *Pg*-ASNase II activity. **(c)** Effect of growth stages for IPTG induction on *Pg*-ASNase II activity. **(d)** A positive correlation between cell growth (line) and enzyme activity (bar chart) was observed. All the values are the means of three replicates ± SD.

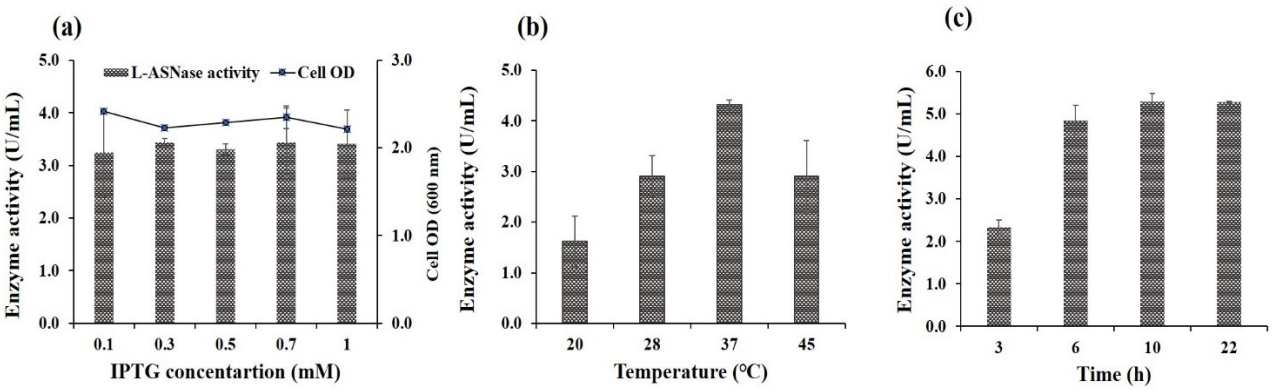

Figure S5. Optimizing the active soluble protein yield of tag-free *Pg*-ASNase II. **(a)** IPTG concentration **(b)** Incubation temperature after induction **(c)** Post induction time.

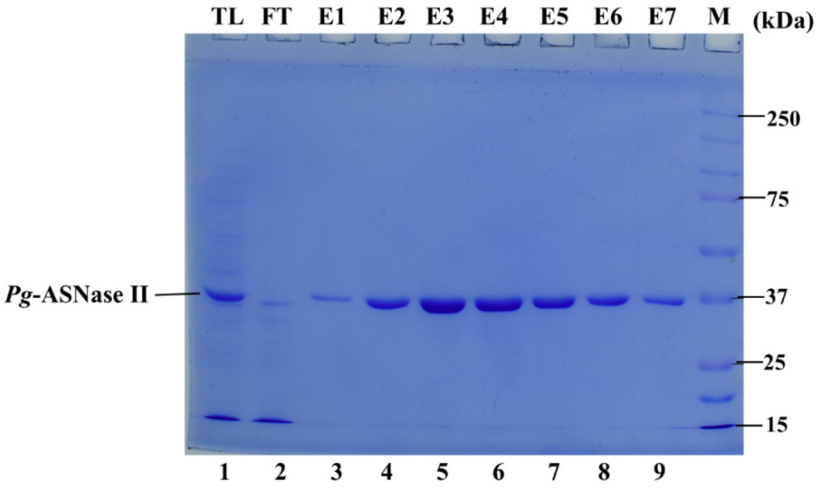

Figure S6. Polyacrylamide gel electrophoresis of tag-free *P*g-ASNase II. 10% SDS-PAGE of purified *Pg*-ASNase II elutions. Lane 1, Total lysate (TL); Lane 2, Flow through (FT); lane 3-9, different elutions (E1-E7) of purified *Pg*-ASNase II; and Lane M, molecular marker.

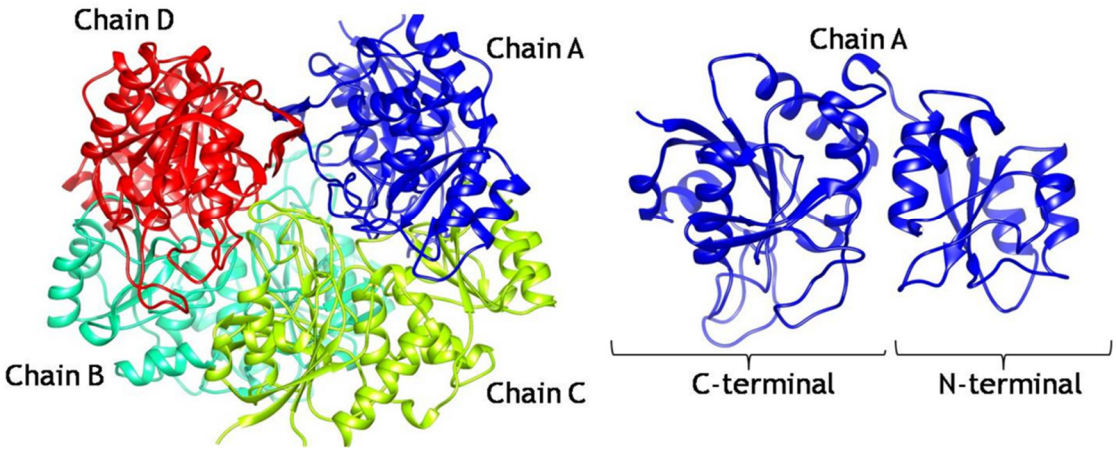

Figure S7. Structure depiction of *Pg*-ASNase II using Swiss Model. Chain A; Blue colour, Chain B; Cyan colour, Chain C; yellow colour, Chain D; Red colour. Structural depiction of monomeric L-ASNase showing two domains, N-terminal and C-terminal for *Pg*-ASNase II.

Table S1. The percentage identity matrix of similarities among five L-ASNase protein sequences with *Pg*-ASNase II sequence.

	UniProt ID	Percent Identity Matrix (%)
Pseudomonas sp. PCH199	-	100
Pseudomonas fluorescens bv. A	O68897.1	93.47
Pseudomonas putida KT2440	Q88K39.1	88.13
Acinetobacter glutaminasificans	P10172.1	63.64
Escherichia coli K-12	P00805.2	47.58
Dickeya chrysanthemi	P06608.1	45.92

Table S2. Acrylamide quantification in raw sample and reduction measures with different enzyme dosages.

Sample	Acrylamide (µg/kg)	Reduction (%)
Control	7131.17	0
1.0 U	5858.37	17
2.5 U	5299.35	25
5.0 U	2108.91	70
10.0 U	2200.97	69