

PEERJ

Molecular docking and functional characterization of β -glucosidase from *Bacillus*

***tequelensis* BD69 expressed in bacterial and yeast heterologous systems**

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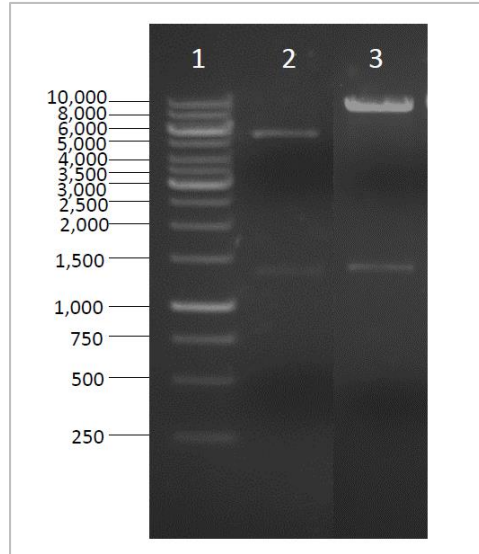


Fig S1. Double digestion of positive clones with EcoRI and NotI; Lane 1: GeneRuler 1-kb DNA ladder (Thermo Scientific); Lane 2: double digested pET-Bteq β gluc; Lane 3: double digested pPIC-Bteq β gluc.

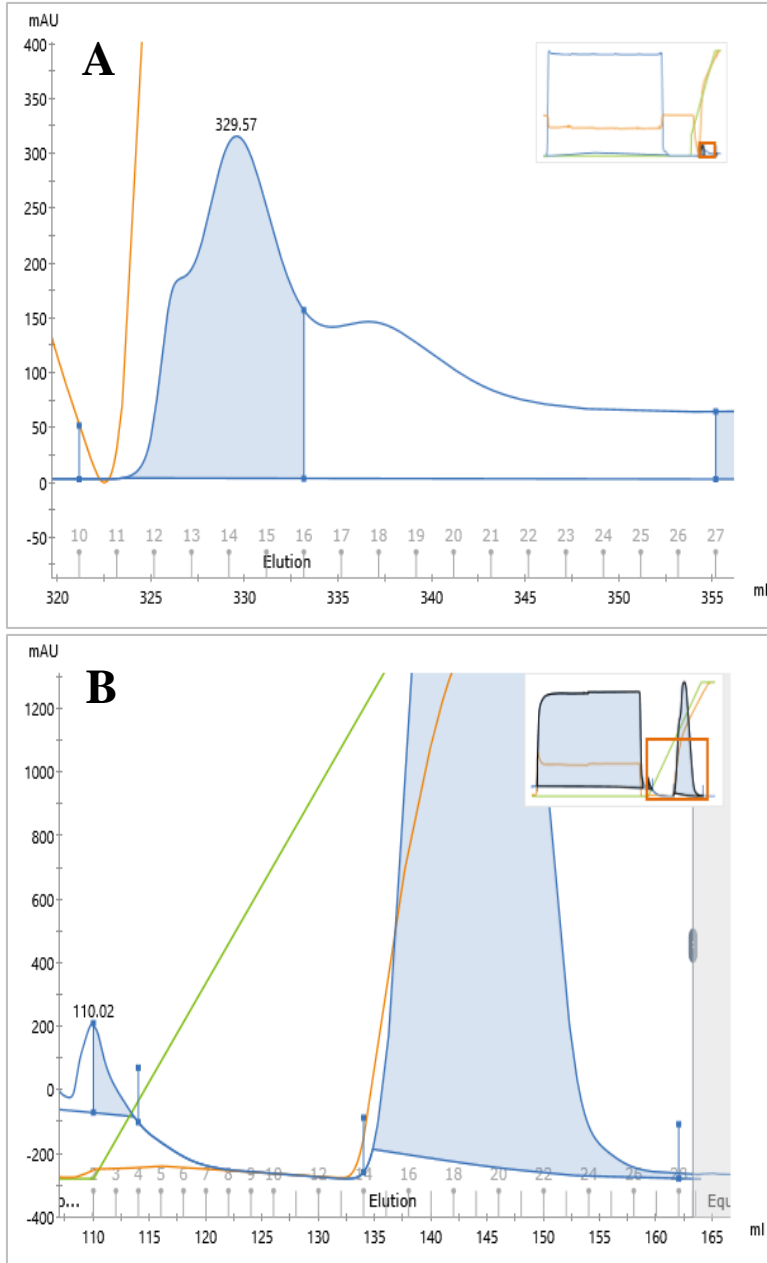


Fig S2. Chromatogram of β -glucosidase purification from AKTA Pure FPLC system (A) pET-Bteq β gluc (B) pPIC-Bteq β gluc.

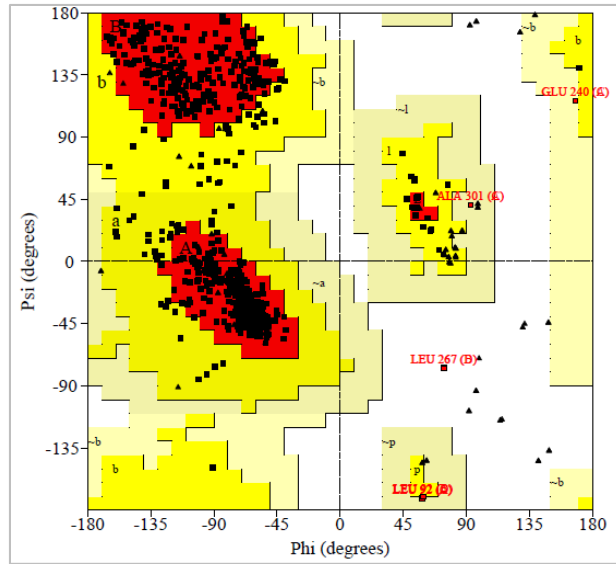


Fig S3. Ramachandran's plot with >90% residues in the most favored regions for structural quality assessment of predicted Bteqβgluc structure.

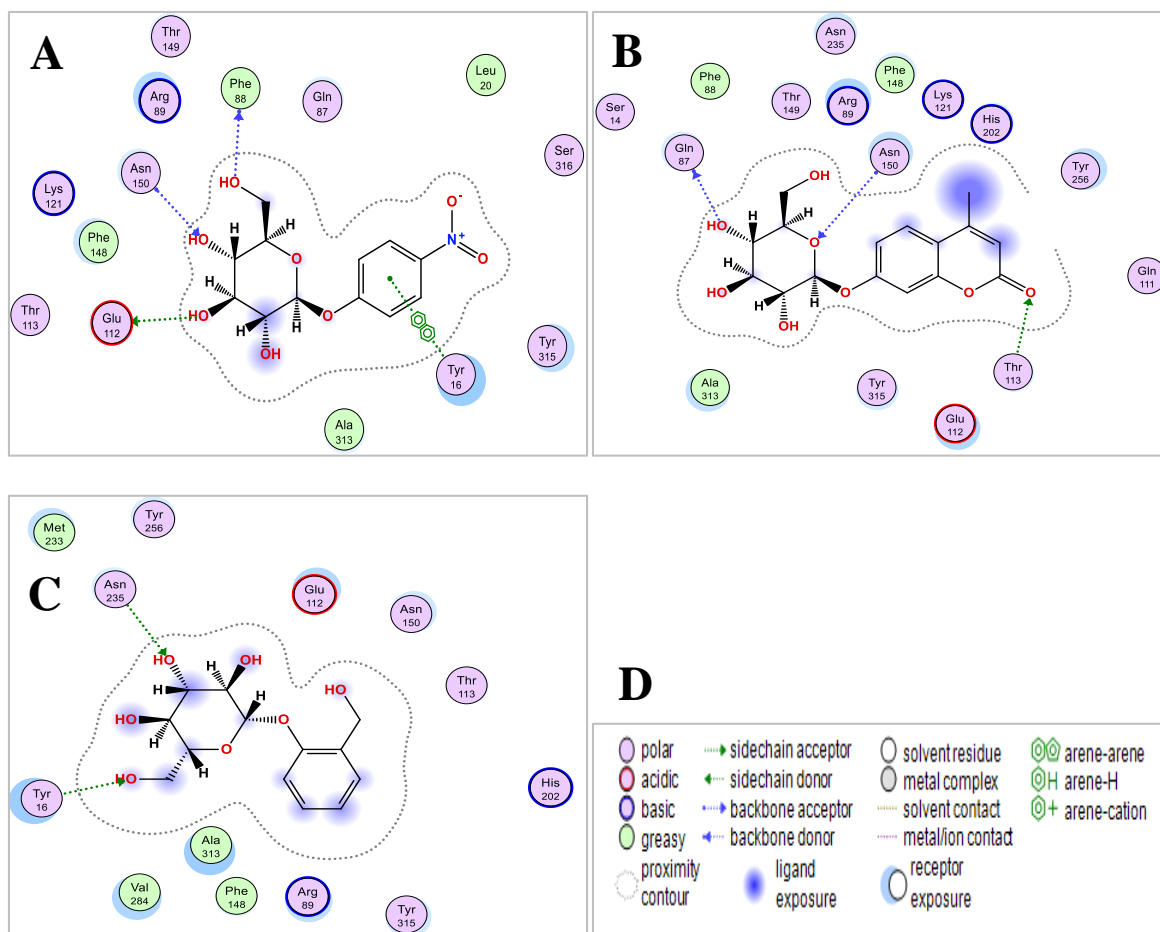
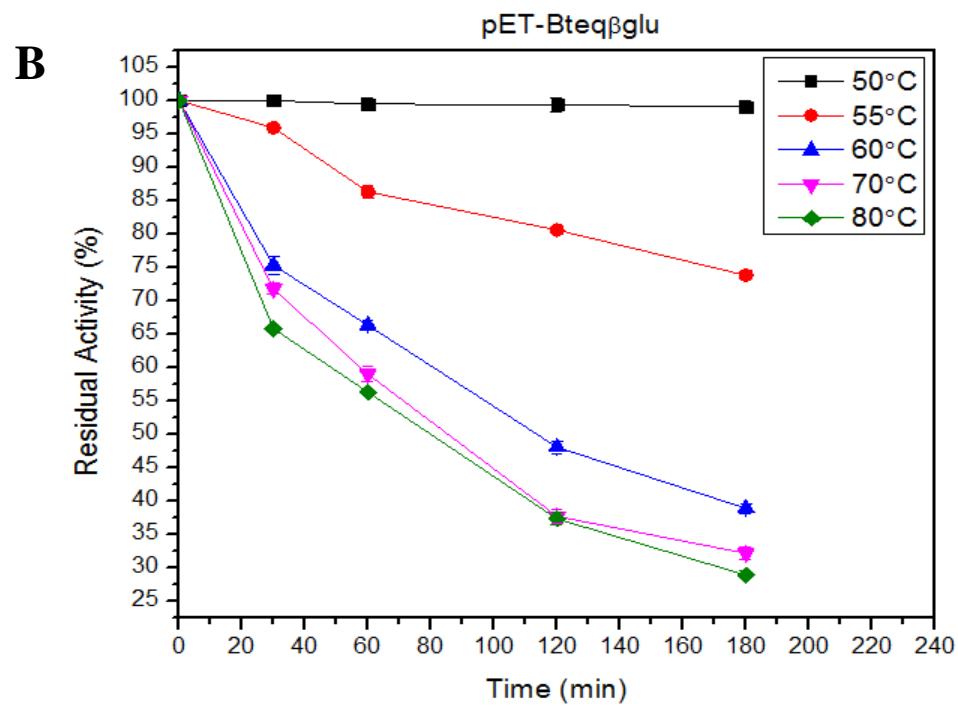
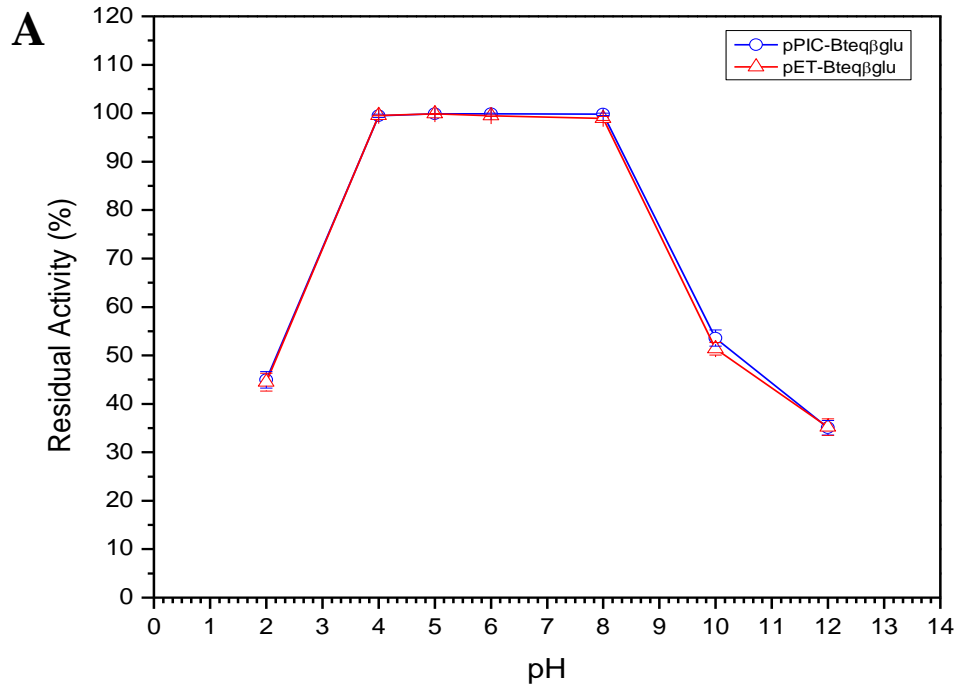


Fig. S4. Two dimensional representation of protein ligand interaction of docking complexes for (A) Bteqβgluc-pNPG, (B) Bteqβgluc-MUG, (C) Bteqβgluc-salicin and (D) legends of interaction diagram.



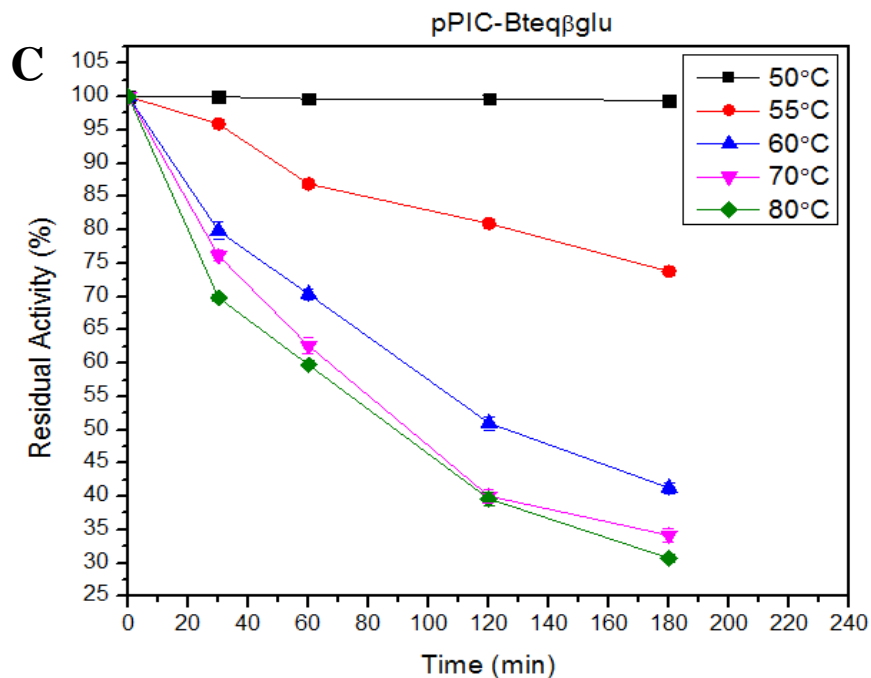


Fig. S5. pH stability (A) of pET-Bteqβgluc and pPIC-Bteqβgluc enzyme determined by pre-incubating at different pH values (2-12) at 50°C for 1 h and thermal-stability of pET-Bteqβgluc (B) pPIC-Bteqβgluc (C) was determined by pre-incubating enzyme at different temperature ranging from 50-80°C.

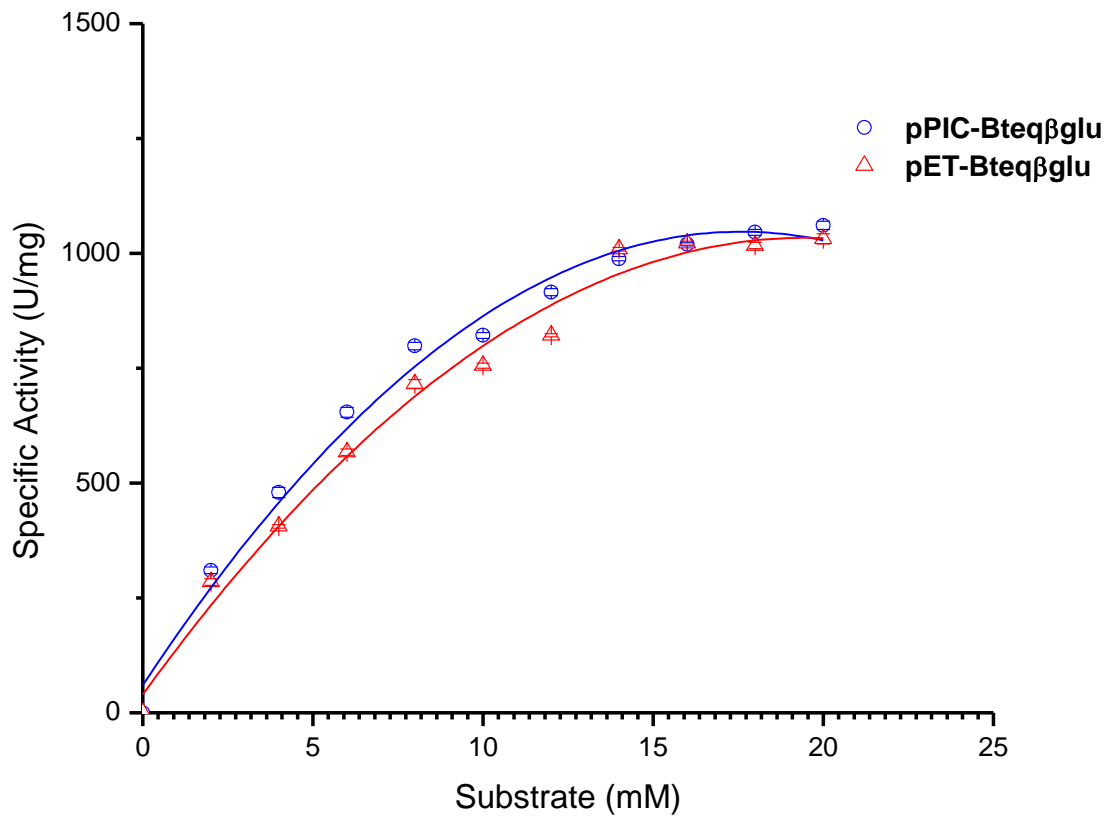


Fig. S6. Non-linear Michaelis-Menten plot of initial reaction velocity (U/mg) versus substrate concentration (mM) at a constant enzyme concentration for pPIC-Bteqβgluc and pET-Bteqβgluc.

Table S1. Summary of Two-way ANOVA test showing interaction effects within and between different substrate concentrations (2-20 mM) and enzyme culture groups (pET-Bteq β gluc and pPIC-Bteq β gluc) for specific activities.

Dependent Variable: Specific activities

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3792862.188 ^a	19	199624.326	3719.180	.000
Intercept	37088675.270	1	37088675.270	690995.264	.000
Groups (pET & pPIC)	32073.775	1	32073.775	597.563	.000
Substrate concentrations	3739327.880	9	415480.876	7740.781	.000
Groups * Sub. con.	21460.534	9	2384.504	44.425	.000
Error	2146.971	40	53.674		
Total	40883684.430	60			
Corrected Total	3795009.160	59			

a. R Squared = .999 (Adjusted R Squared = .999)

Table S2. Summary of Two-way ANOVA test showing interaction effects within and between different temperatures (30-80°C) and enzyme culture groups (pET-Bteq β gluc and pPIC-Bteq β gluc) for specific activities.

Dependent Variable: Specific activities

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6102759.597 ^a	17	358985.859	4425.912	.000
Intercept	22733965.084	1	22733965.084	280285.524	.000
Groups (pET & pPIC)	37890.236	1	37890.236	467.146	.000
Temperature	6043385.327	8	755423.166	9313.561	.000
Groups * Temp.	21484.034	8	2685.504	33.109	.000
Error	2919.961	36	81.110		
Total	28839644.641	54			
Corrected Total	6105679.558	53			

a. R Squared = 1.000 (Adjusted R Squared = .999)

Table S3. Summary of Two-way ANOVA test showing interaction effects within and between different pH (4-10) and enzyme culture groups (pET-Bteqβgluc and pPIC-Bteqβgluc) for Log10 V_{max} .

Dependent Variable: Log10 V_{max}

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.795 ^a	7	.114	1051.464	.000
Intercept	192.927	1	192.927	1786144.502	.000
Groups (pET & pPIC)	.004	1	.004	37.215	.000
pH	.777	3	.259	2396.707	.000
Groups * pH	.014	3	.005	44.303	.000
Error	.002	16	.000		
Total	193.724	24			
Corrected Total	.797	23			

a. R Squared = .998 (Adjusted R Squared = .997)