Anoxybacillus rupiensis TS-4 α-amylase: A thermostable amylase exhibiting prominent application in the detergent industry

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Abstract

The physicochemical conditions influencing α-amylase secretion by a thermophilic bacterium, *Anoxybacillus rupiensis* TS-4 (Genbank Number, KU360725) were optimized by the response surface methodology, using Plackett Burman design, followed by Box Behnken Design to enhance amylase production by 3 fold as compared to the one variable at a time approach. The trends revealed incubation temperature, medium pH and starch concentration as the most significant variables. The amylase was purified by ion exchange chromatography, followed by size exclusion chromatography with fold purification and yield of 17.85 and 34.72%, respectively. The molecular weight, K_m and V_{max} of the purified amylase were 48 kD, 0.58 mgmL^{-1} and 3124 μmolmL^{-1} min^{-1}, respectively. It catalyzed starch over a broader range of temperature and pH, having optima as 80 ˚C and 8, respectively. The enzyme was stable at a broad range of temperatures and pH, displaying higher half-life and reduced deactivation rate constant. The feasibility of the starch catalysis reaction mediated by the studied amylase was substantiated by determining the thermodynamic parameters, such as alterations in the enthalpy, entropy, activation energy and Gibb's free energy. The attributes of the amylase such as calcium independence, alkalitolerance and stability in presence of various chelators and surfactants aid uniqueness, novelty and commercial promise.

Production and purification of *A. rupiensis* TS-4 α-amylase

Table 1 Experimental design used to optimize physicochemical factors affecting *A. rupiensis* TS-4 amylase production by the Box Behnken Design (Software used was Sigma XL, USA)

Run Order	Temperature (˚C)	pH	Starch (% w/v)	Response (Y)	
				Experimental (U/ ml)	Predicted (U/ ml)
1	50	8	0.1	0	0
2	50	6	2	70	70.17
3	60	6	1	65	69.37
4	50	8	2	80	83.75
5	60	7	0.1	0	0
6	50	6	0.1	0	0
7	50	7	1	95	97.99
8	40	6	1	70	69.37
9	50	7	1	95	97.99
10	60	8	1	80	80.62
11	60	7	2	90	85.72
12	50	7	1	95	97.99
13	40	7	2	75	75.62
14	40	7	0.1	0	0
15	40	8	1	80	77.87

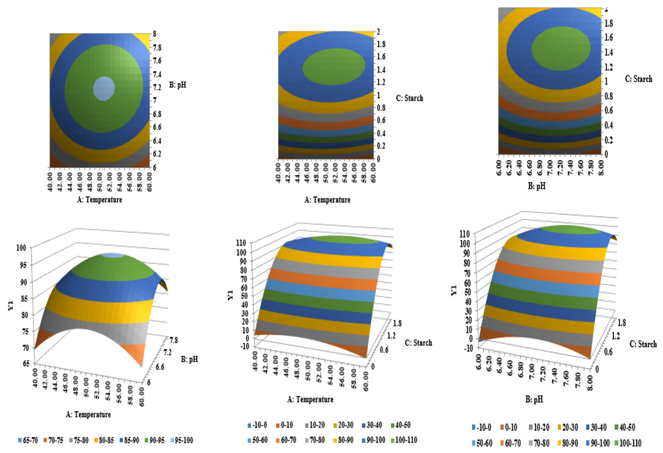


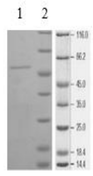
Table 2 Statistical analysis of the experiments employed to optimize physicochemical factors affecting *A. rupiensis* TS-4 amylase production by the Box Behnken Design

Source	Degree of freedom	Sum of square	Mean square	F-value	P value > F
Model	9	20517	2279.7	107.28	0.0000
Error	5	106.25	21.250		
Lack of Fit	3	106.25	35.417		0.0000
Pure Error	2	0	0		
Total (Model + Error)	14	20623	1473.1		

Where, R^2 (experimental): 0.99; R^2 (adjusted): 0.98; Coefficient of variance: 4.61

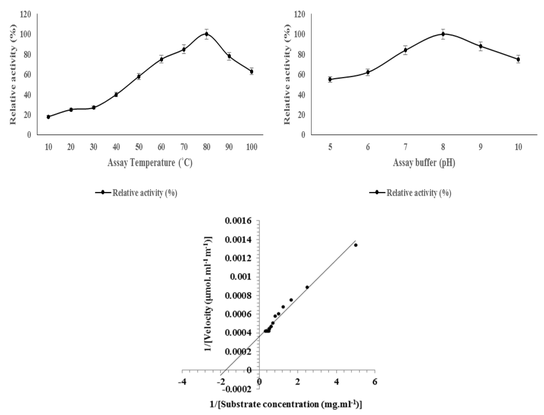
Table 3 Purification profile along with the fold purification and purification yield

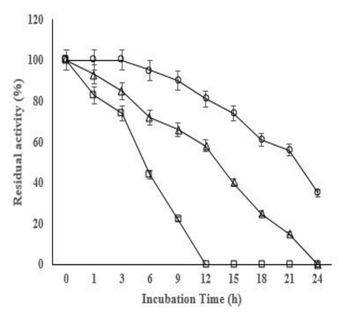
Purification Steps	Volume (mL)	Total Activity (U)	Total Protein (mg)	Specific Activity (U/mg)	Purification fold	Yield (%)
Crude enzyme	100	11800	23.6	500	-	100
0-50% fraction	4	721	1.31	550	1.1	6.11
50-70% fraction	4	3147	1	3147	6.3	26.67
Ion Exchange Chromatography	4	4523	0.52	8625	17.25	38.33
Size Exclusion Chromatography	4	4097	0.45	8928	17.85	34.72



SDS-PAGE (12%) gel eletrophoresis;
Lane 1 (purified α-amylase)
Lane 2 (Protein Marker, Thermo Fisher)

Characterization of *A. rupiensis* TS-4 α-amylase





Temperature stability profiles of *A. rupiensis* TS-4 amylase; 50 °C (Circle), 70 °C (Triangle) and 90 °C (Square)

Thermodynamics, Detergent applications of *A. rupiensis* TS-4 α-amylase

Table 4 Calculation of various thermodynamic parameters of enzyme-substrate reaction

Conditions	K_d (m^{-1})			$T_{1/2}$ (h)			ΔG* (kJ/ mol)			ΔH* (kJ/mol)	ΔS* (J/mol K)	E (kJ/mol)
	50 ˚C	70 ˚C	90 ˚C	50 ˚C	70 ˚C	90 ˚C	50 ˚C	70 ˚C	90 ˚C			
Without $CaCl_2$	0.000525	0.000888	0.002310	22	13	5	101.4	105.1	108.7	45.86	-171.54	49.63
20 mM $CaCl_2$	0.00578	0.001050	0.003850	20	11	3	101.5	104.1	106.6	60.23	-127.86	64.01
pH 6	0.000578	0.001283	0.003850	20	9	3	101.3	103.9	106.5	58.93	-131.12	62.71
pH 7	0.000525	0.000888	0.002310	22	13	5	101.4	105.1	108.7	45.86	-171.54	49.63
pH 8	0.000679	0.001283	0.003850	17	9	3	101.1	103.5	106.2	54.20	-144.27	57.99
pH 9	0.000772	0.001444	0.005770	13	8	2	100.8	103.7	106.5	60.30	-124.52	63.84

Table 5 Washing efficiency of *A. rupiensis* TS-4 amylase with various commercially available detergents; whereas, 0.2% surfactant and detergent concentration, 1000 μL amylase in 25 mL of 20 mM tris-HCl buffer, pH 8 was used

Experiments	Washing conditions Volume: 25 mL Incubation Temperature: 70 ˚C Time duration: 1 h	Starch (mg/ mL) [S_2]	Washing efficiency (%) [$(S_1 - S_2 / S_1) \times 100\%$]
Initial starch concentration [S_1]: 50 mg/ mL			
Control	Buffer	46.33	7.34
Test 1	Buffer + SDS	33.67	32.67
Test 2	Buffer + Tween 20	42.80	14.40
Test 3	Buffer + Tween 80	37.71	24.58
Test 4	Buffer + Triton 100	35.29	29.42
Test 5	Buffer + Surf excel	31.42	37.16
Test 6	Buffer + Tide	32.07	35.86
Test 7	Buffer + Rin	34.03	31.94
Test 8	Buffer + Ghadi	35.49	29.02
Test 9	Buffer + Aerial	24.61	49.22
Test 10	Buffer + Wheel	29.71	40.58
Test 11	Buffer + Enzyme	24.30	51.40
Test 12	Buffer + Enzyme + SDS	29.11	41.78
Test 13	Buffer + Enzyme + Tween 20	30.25	39.50
Test 14	Buffer + Enzyme + Tween 80	27.44	45.12
Test 15	Buffer + Enzyme + Triton 100	32.29	35.42
Test 16	Buffer+ Enzyme + Surf excel	22.48	55.04
Test 17	Buffer + Enzyme + Tide	29.46	41.08
Test 18	Buffer + Enzyme + Rin	24.17	51.67
Test 19	Buffer + Enzyme + Ghadi	30.82	38.36
Test 20	Buffer + Enzyme + Aerial	18.09	63.82
Test 21	Buffer + Enzyme + Wheel	24.93	50.14

Table 6 Comparative profile of *A. rupiensis* TS-4 amylase with other reported amylases of *Anoxybacillus* sp.

Organism	Molecular weight (kDa)	Temperature optima	pH optima	Stability attributes	Reference
***Anoxybacillus* sp. AH1**	85	60	7	60% activity retained after 120 min at 60	D'Amico et al., 2003
***A. flavithermus* SO-19**	96	70	6	62% activity retained after 720 min at 50	Tari et al., 2008
A. amylolyticus	60	60	5.6	50% activity retained after 65h at 60	Fincan et al., 2014
***Anoxybacillus* sp. KP1**	-NA-	60	8	Stable between 50 and 70 for 30 min	Acher et al., 2016
A. flavithermus	60	55	6	50% activity retained after 120 min at 50	Ozdemir et al., 2016
***Anoxybacillus* sp. ASKA**	50	60	8	50% activity retained after 48 h at 65	Muhammad et al., 2017
***A. rupiensis* TS-4**	48	80	8	50% activity retained after 22 h at 50	Present study

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