



Figure 1: *Bacillus thuringiensis* showing zone of clearance on skim milk agar plate.



Figure 2: *Pseudomonas aeruginosa* showing zone of clearance on Skim milk agar plate.



Figure 3: Protease Production Medium broth inoculated with *Pseudomonas aeruginosa* (left) and *Bacillus thuringiensis* (right) for protease estimation.



Figure 4: Protease Production Medium with *Bacillus thuringiensis* studied for optimization of pH, temperature, salt concentration before incubation.

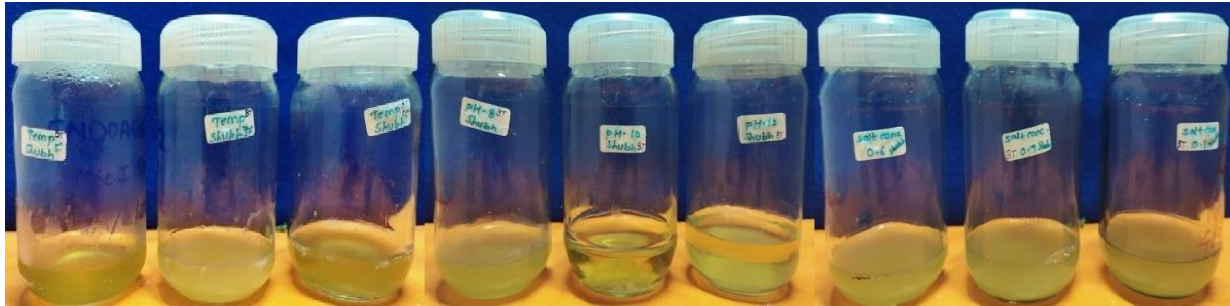


Figure 5: Protease Production Medium with *Bacillus thuringiensis* studied for optimization of pH, temperature, salt concentration after incubation.



Figure 6: Protease Production Medium with *Pseudomonas aeruginosa* for optimization of pH, temperature, salt concentration before incubation.

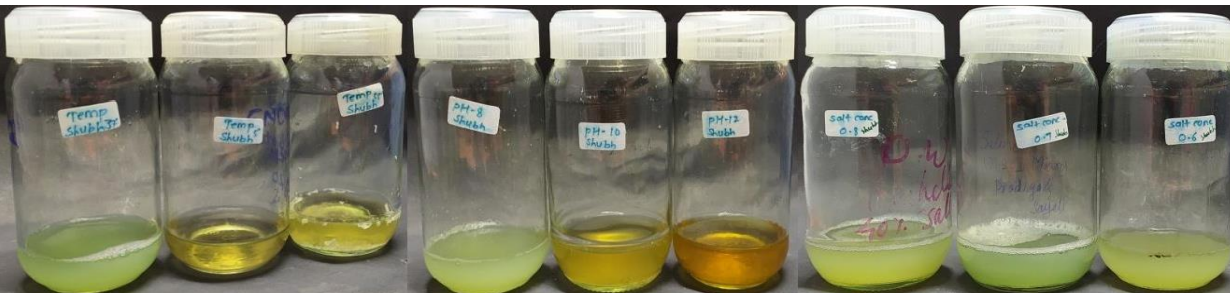


Figure 7: Protease Production Medium with *Pseudomonas aeruginosa* for optimization of pH, temperature, salt concentration after incubation.

Starting percent saturation	Final percent saturation to be obtained																
	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0	113	144	176	208	242	277	314	351	390	430	472	516	561	608	657	708	761
5	85	115	146	179	212	246	282	319	358	397	439	481	526	572	621	671	723
10	57	86	117	149	182	216	251	287	325	364	405	447	491	537	584	634	685
15	28	58	88	119	151	185	219	255	293	331	371	413	456	501	548	596	647
20	0	29	59	89	121	154	188	223	260	298	337	378	421	465	511	559	609
25		0	29	60	91	123	157	191	228	265	304	344	386	429	475	522	571
30			0	30	61	92	125	160	195	232	270	309	351	393	438	485	533
35				0	30	62	94	128	163	199	236	275	316	358	402	447	495
40					0	31	63	96	130	166	202	241	281	322	365	410	457
45						0	31	64	98	132	169	206	245	286	329	373	419
50							0	32	65	99	135	172	210	250	292	335	381
55								0	33	66	101	138	175	215	256	298	343
60									0	33	67	103	140	179	219	261	305
65										0	34	69	105	143	183	224	267
70											0	34	70	107	146	186	228
75												0	35	72	110	149	190
80													0	36	73	112	152
85														0	37	75	114
90															0	37	76
95																0	38

Figure 8: Ammonium sulfate precipitation chart [25].

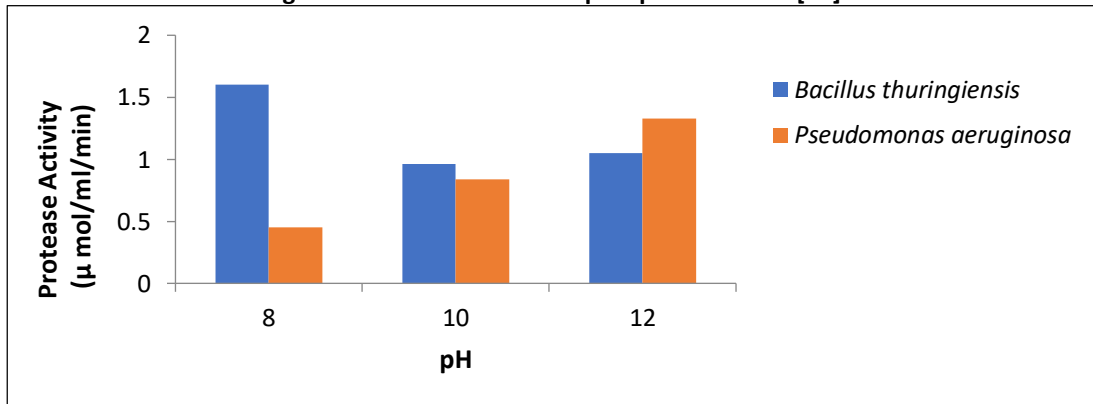


Figure 9: Effect of pH on crude protease activity.

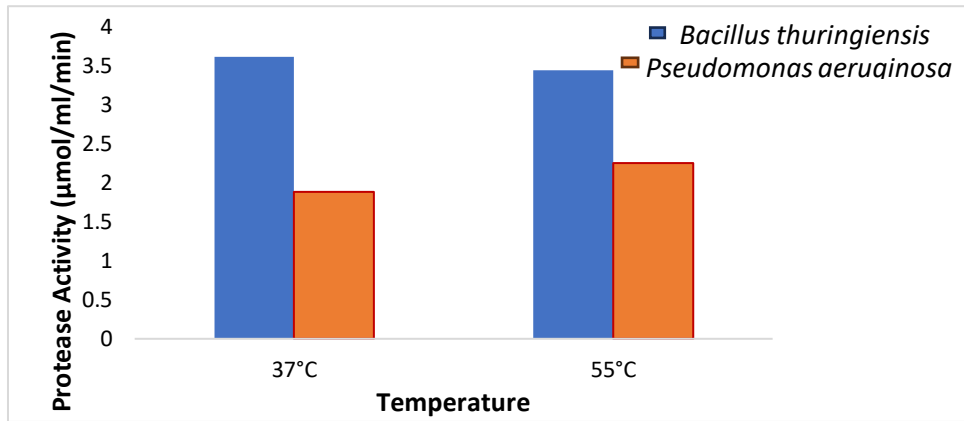


Figure 10: Effect of temperature on crude protease activity

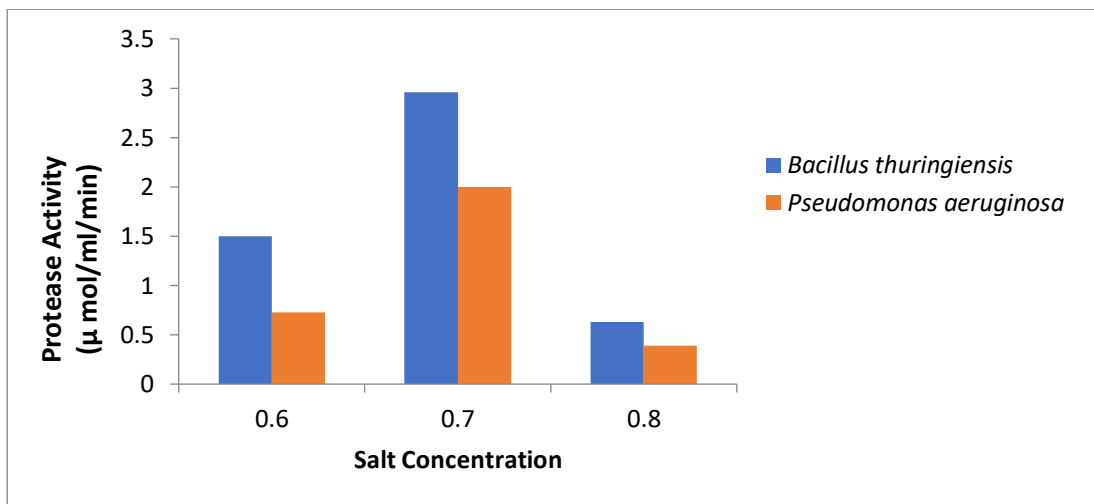


Figure 11: Effect of salt concentration on crude protease activity.

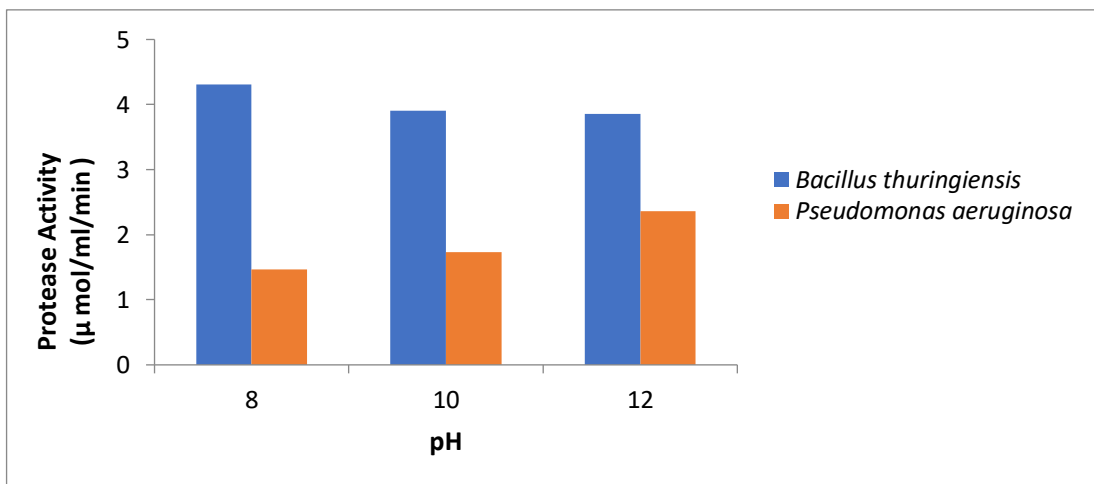


Figure 12: Effect of pH on Protease Activity at 80% Ammonium Sulfate Saturation.

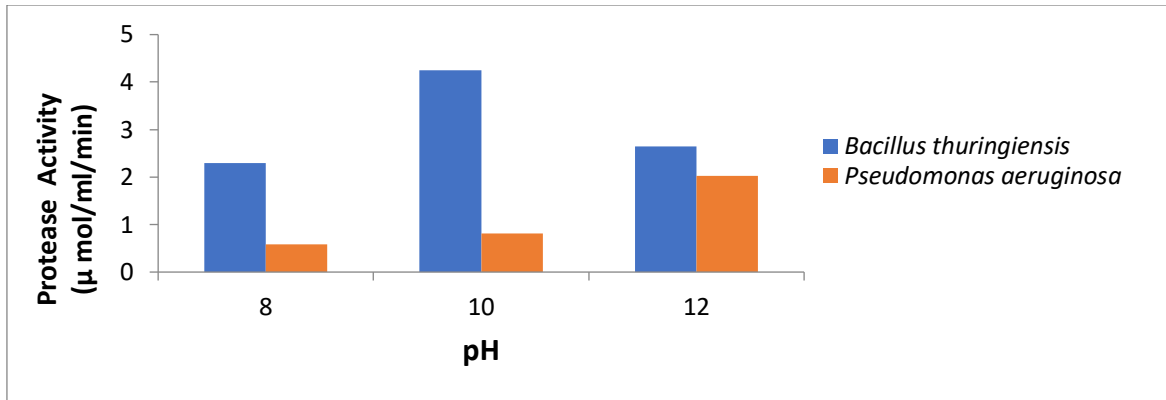


Figure 13: Effect of pH on Protease Activity at 100% Ammonium Sulfate Saturation.

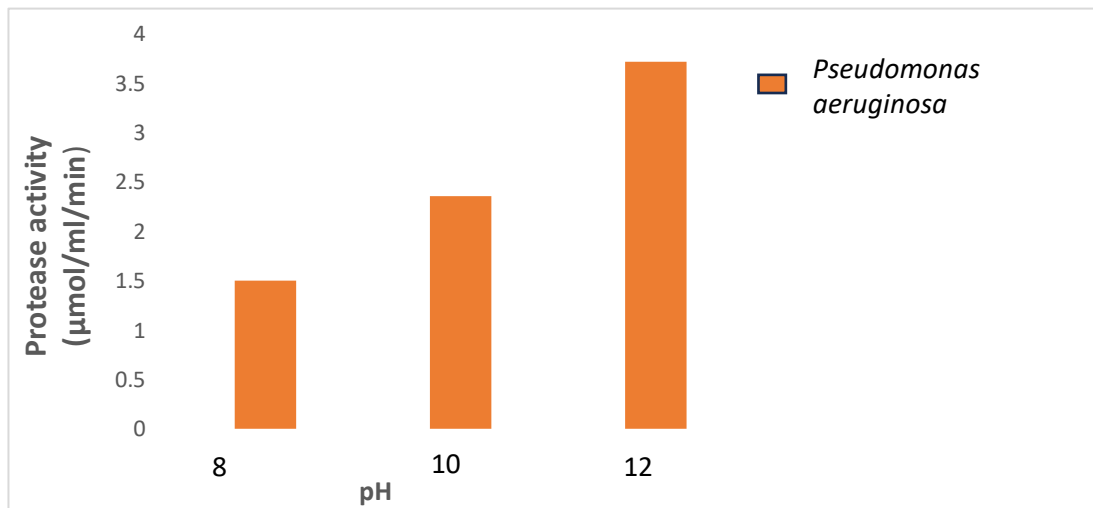


Figure 14: Effect of pH on Protease Activity at 60% Ammonium Sulfate Saturation

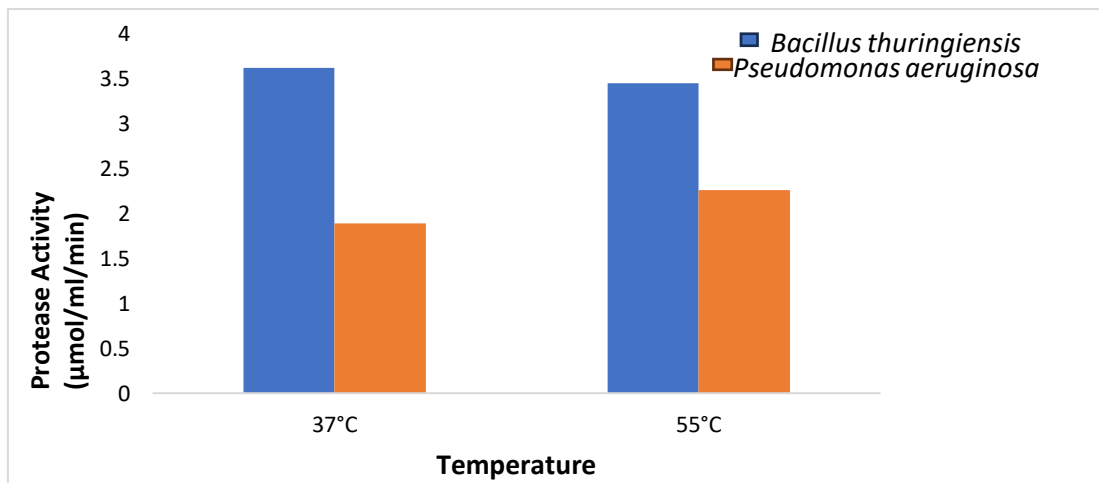


Figure 15: Effect of Temperature on Protease Activity at 80% Ammonium Sulfate Saturation

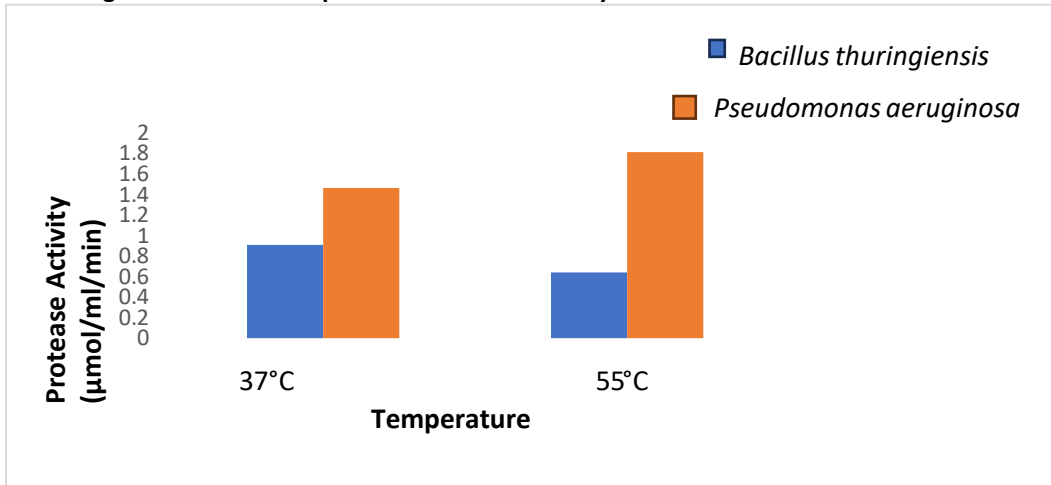


Figure 16: Effect of Temperature on Protease Activity at 100% Ammonium Sulfate Saturation.

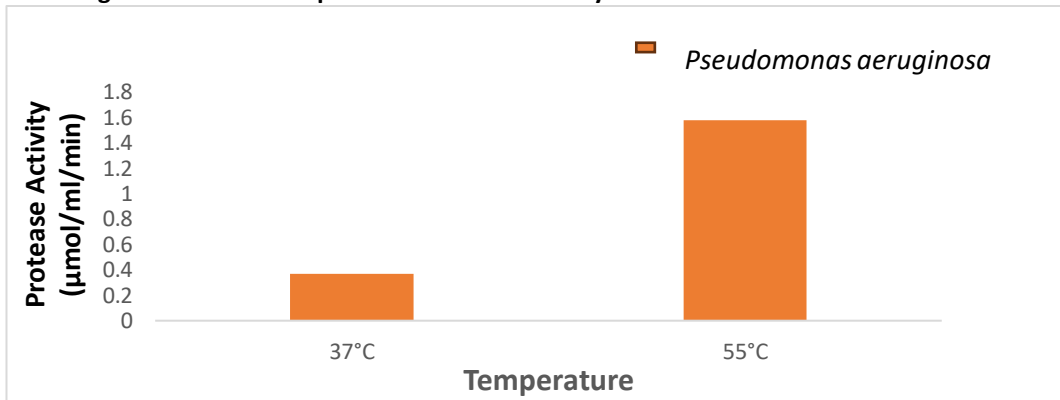


Figure 17: Effect of Temperature on Protease Activity at 60% Ammonium Sulfate Saturation.

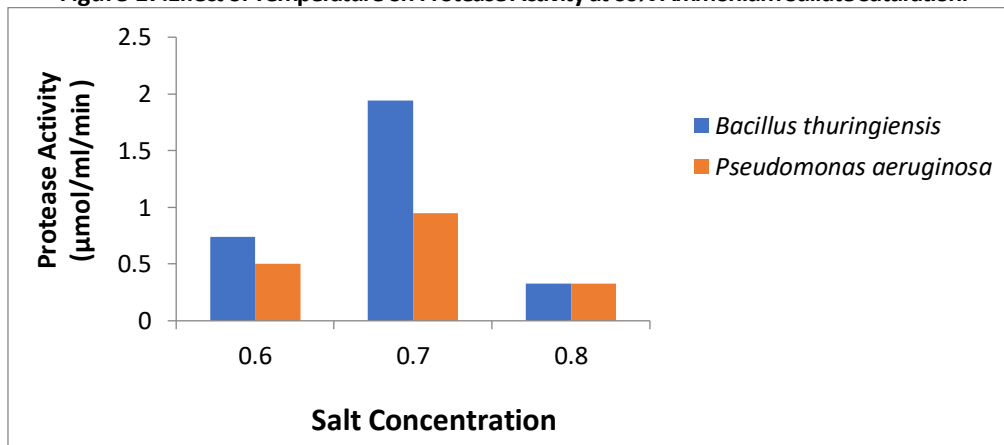


Figure 18: Effect of salt concentration on Protease Activity at 80% Ammonium Sulfate Saturation.

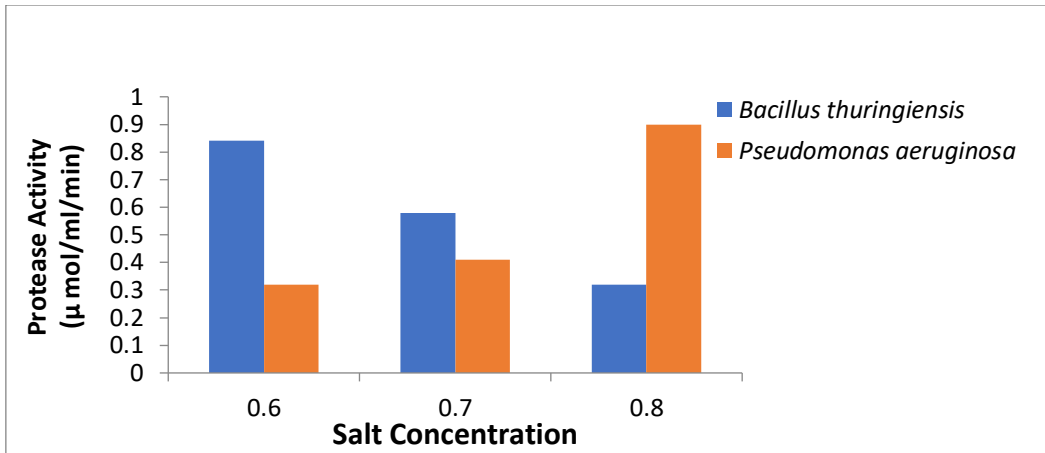


Figure 19: Effect of salt concentration on Protease Activity at 100% Ammonium Sulfate Saturation.

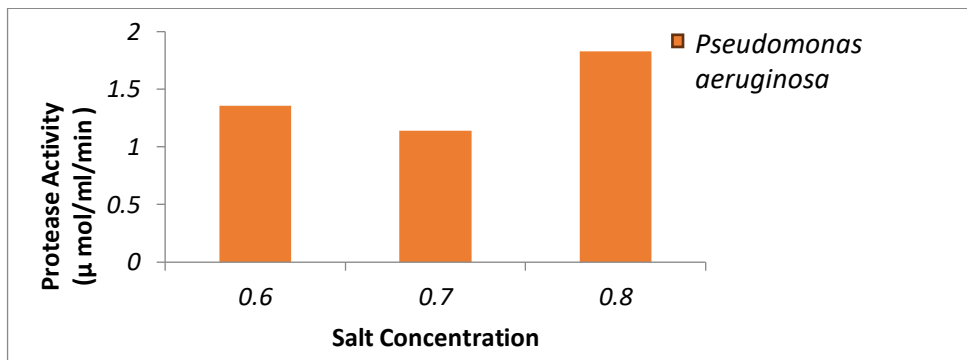
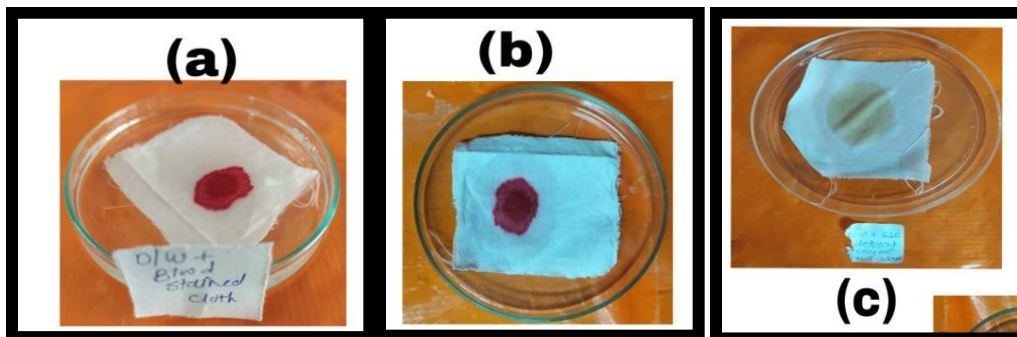


Figure 20: Effect of salt concentration on Protease Activity at 60% Ammonium Sulfate Saturation.



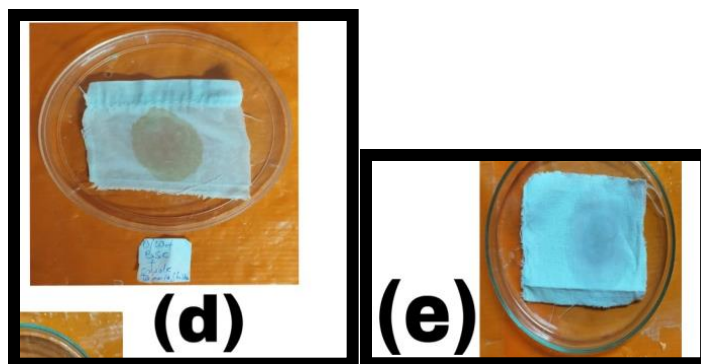


Figure 21: Blood stain removal activity of protease from *Bacillus thuringiensis*. a. A tray containing 100 mL of distilled water and a blood-stained cloth (control). b. A tray containing 100 mL of distilled water, a blood-stained cloth, and 2 mL of protease enzyme. c. Tray with distilled water (100 mL) + blood-stained cloth + 2 mL of commercial detergent-Tide-5mg/mL+ 1 mL of protease enzyme. d. A tray containing 100 mL of distilled water, a blood-stained cloth, and 2 mL of commercial detergent (Tide 5 mg/mL). e. A tray containing 100 mL of distilled water, a blood-stained cloth, 2 mL of commercial detergent (Tide 5 mg/mL) and 2 mL of protease enzyme.

Table 1: Purification table for the protease from *Pseudomonas aeruginosa*.

Purification step		Volume (ml)	Total Protein (mg)	Total activity	Specific activity (Units/mg)	Purification yield (%)	Fold Purification
Salt Optimization							
Ammonium Sulfate salt (grams)	Ammonium Sulfate Saturation (%)						
0.8	60%	50 ml	12	21.98	1.83	0.083	3.51
0.8	80%	50 ml	37	52.77	1.42	0.026	2.73
0.6	100%	50 ml	34	10.99	0.32	0.029	0.61
0.7	60%	50 ml	23	26.38	1.14	0.043	2.19
0.7	80%	50 ml	46	43.97	0.95	0.021	1.82
0.7	100%	50 ml	64	26.38	0.41	0.015	0.78
0.6	60%	50 ml	21	28.58	1.36	0.047	2.61
0.6	80%	50 ml	26	13.19	0.50	0.037	0.96
0.8	100%	50 ml	51	46.17	0.90	0.019	1.73

Purification Step	Volume	Total	Total	Specific	Purification	Fold
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pH Optimization		(ml)	Protein (mg)	activity	activity(Units/mg)	n yield(%)	Purification
pH	Ammonium Sulfate Saturation (%)						
8	60%	50 ml	49	61.56	1.25	0.02	2.4
8	80%	50 ml	58	85.75	1.47	0.017	2.82
8	100%	50 ml	67	39.58	0.59	0.014	1.13
10	60%	50 ml	39	92.35	2.36	0.025	4.53
10	80%	50 ml	52	90.15	1.73	0.019	3.32
10	100%	50 ml	65	52.77	0.81	0.015	1.55
12	60%	50 ml	23	85.75	3.72	0.043	7.15
12	80%	50 ml	26	61.56	2.36	0.038	4.53
12	100%	50 ml	37	74.76	2.02	0.027	3.88

Purification Step		Volume (ml)	Total Protein (mg)	Total activity	Specific activity (Units/mg)	Purification yield(%)	Fold Purification
Temperature Optimization							
Temperature	Ammonium Sulfate Saturation (%)						
37°C	60%	50 ml	65	24.18	0.37	0.015	0.71
37°C	80%	50 ml	52	10.99	0.21	0.019	0.40
37°C	100%	50 ml	24	35.18	1.46	0.041	2.80
55°C	60%	50 ml	61	96.75	1.58	0.016	0.03
55°C	80%	50 ml	29	39.58	1.36	0.034	2.61
55°C	100%	50 ml	17	30.78	1.81	0.058	3.48

Purification step	Volume (ml)	Total Protein (mg)	Total activity	Specific activity (Units/mg)	Purification yield(%)	Fold Purification
Optimization of the crude enzyme						
pH 8	50 ml	39	17.59	0.45	0.025	0.86
pH 10	50 ml	52	44.15	0.84	0.019	1.61
pH 12	50 ml	23	30.78	1.33	0.043	2.55
Temperature 37°C	50 ml	43	81.35	1.89	0.023	3.63
Temperature 55°C	50 ml	31	70.36	0.46	0.032	4.34
Salt concentration 0.6%	50 ml	42	30.78	0.73	0.023	1.40
Salt concentration 0.7%	50 ml	45	90.15	2.00	0.022	3.84
Salt concentration 0.8%	50 ml	28	10.99	0.39	0.035	0.75

Table 2: Purification table for the protease from *Bacillus thuringiensis*.

Purification Step	Volume (ml)	Total Protein	Total activity	Specific activity(Units/mg)	Purification yield(%)	Fold purification
Salt Optimization						

Ammonium Sulfate salt (grams)	Ammonium Sulfate Saturation (%)		(mg)				
0.8	100%	50 ml	68	22.07	0.324	0.014	0.17
0.8	80%	50 ml	72	24.28	0.337	0.013	0.176
0.7	100%	50 ml	75	44.15	0.588	0.013	0.31
0.7	80%	50 ml	25	48.56	1.94	0.039	1.037
0.6	100%	50 ml	52	44.15	0.84	0.019	0.44
0.6	80%	50 ml	86	64.02	0.74	0.011	0.39

Purification Step		Volume (ml)	Total Protein (mg)	Total activity	Specific activity (Units/mg)	Purification yield(%)	Fold purification
pH Optimization							
pH	Ammonium Sulfate Saturation (%)						
8	80%	50 ml	23	99.34	4.31	0.043	2.30
8	100%	50 ml	19	44.15	2.3	0.052	1.22
10	80%	50 ml	22	86.09	3.91	0.045	2.09
10	100%	50 ml	14	59.6	4.25	0.071	2.27
12	80%	50 ml	16	61.81	3.86	0.062	2.06
12	100%	50 ml	20	52.98	2.64	0.049	1.41

Purification Step		Volume (ml)	Total Protein (mg)	Total activity	Specific activity (Units/mg)	Purification yield(%)	Fold purification
Temperature Optimization							
Temperature	Ammonium Sulfate Saturation (%)						
37°C	80%	50 ml	23	85.75	3.7	0.043	1.97
37°C	100%	50 ml	29	26.49	0.91	0.034	0.48
55°C	80%	50 ml	37	74.76	2.02	0.027	1.08
55°C	100%	50 ml	31	19.86	0.64	0.032	0.34

Purification step	Volume (ml)	Total Protein (mg)	Total activity	Specific activity (Units/mg)	Purification yield (%)	Fold Purification
Optimization of the crude enzyme						
pH 8	50 ml	11	17.66	1.6	0.09	0.85
pH 10	50 ml	25	24.24	0.96	0.037	0.51
pH 12	50 ml	23	24.24	1.05	0.043	0.56

Salt concentration 0.6%	50 ml	22	33.11	1.5	0.045	0.80
Salt concentration 0.7%	50 ml	29	86.09	2.96	0.034	1.58
Salt concentration 0.8%	50 ml	28	17.66	0.63	0.035	0.33
Temperature 55°C	50 ml	23	79.47	3.45	0.043	1.84
Temperature 37°C	50 ml	14	50.77	3.62	0.071	1.93