

APPENDIX I**G-MEDIUM**

Yeast extract	4.0g
Protease peptone	4.0g
NaCl	5.6g
K ₂ HPO ₄	5.6g
KH ₂ PO ₄	1.2g
MgSO ₄	0.3g
MnSO ₄	0.02g
FeSO ₄	0.02g
Glucose (w/v)	0.5%

The ingredients are dissolved in 1L of distilled water and autoclaved at 15 psi for 15 minutes. Glucose was autoclaved separately and added to the medium later.

APPENDIX II

Reagents used for analytical studies

(i) Reducing sugar

(a) DNS solution:

10g Dinitrosalicylic acid

2g Phenol

10g NaOH

0.5g Na_2SO_4

1 litre Distilled water

(b) Rochelle salt

40g Potassium sodium tartarate in 1L distilled water

(ii) Protein

Lowry Reagent, modified

Sodium deoxycholate, 1.5 mg/ml (DOC solution)

Folin and Ciocalteus, Phenol reagent

Protein standard; BSA, 400 $\mu\text{g}/\text{mg}$

(iii) Protease assay

Azocasein solution (pH 8.3), 5 mg/ml

Trichloroacetic acid 5% (w/v)

10N NaOH

(iv) Alkaline phosphatase

p-nitrophenylphosphate : 1.0 mM in 1M Tris buffer, pH 8

p-nitrophenol : 50 µg/ml of Tris buffer, pH 8

(v) Pyruvate carboxylase

Tris (hydroxymethyl) aminomethanol - HCl buffer, 0.1M, pH 7.2

Magnesium pyruvate, 0.1M

Potassium bicarbonate, 0.1M

NADH, 10 mM

ATP, 0.1M, pH 7.2

Serum albumin, 50 mg/ml

Malate dehydrogenase, 0.5 mg suspended in 1.0 ml of 2.8M Ammonium persulfate solution, specific activity 720 units/mg.

(vi) Isocitrate dehydrogenase

1mM EDTA

0.3 mM Dithiothreitol acid (DTT)

100 mM Tris-HCl buffer, pH 7.4

20 mM MnSO₄

1.5 mM NADP

0.3 mM NADPH

(vii) Citrate synthase

Tris[(hydroxymethyl)aminimethanel] - HCl, 0.5M, pH 8.0

DTNB, 2.5 mM, dissolved in 20 mM Tris-HCl, pH 8.0

Oxaloacetate, 2 mM, freshly prepared and neutralized

Acetyl-CoA, 1 mM

APPENDIX III

Procedure of Analysis of Reducing Sugar and Protein

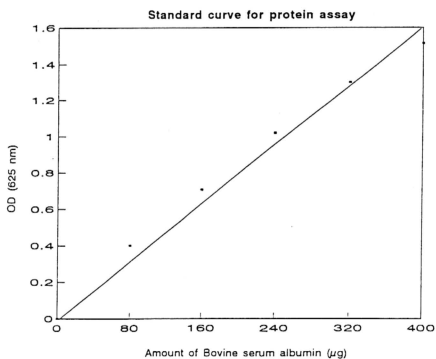
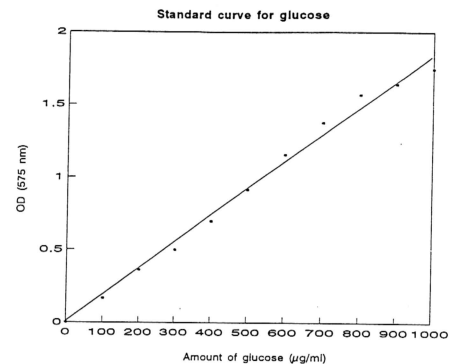
(i) Reducing sugar

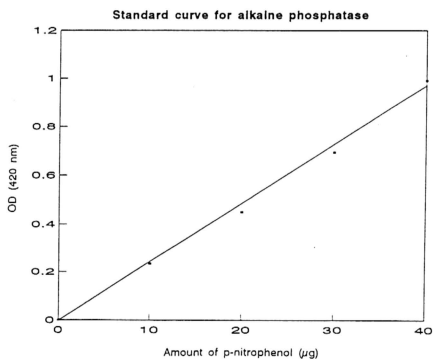
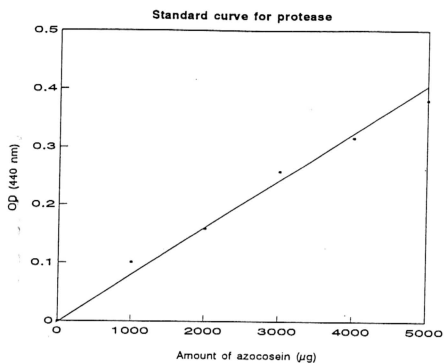
An aliquot consisting of 3 ml of an approximately diluted sample was mixed with 3 ml of DNS solution. The mixture was heated for 15 min in a boiling waterbath which was then adjusted to ambient temperature. To this, 1 ml of Rochelle salt was added. The colour intensity was measured at 575 nm by a Shimadzu spectrophotometer. Reducing sugar was determined against a standard curve prepared with glucose at concentrations of 100 to 1000 $\mu\text{g/ml}$.

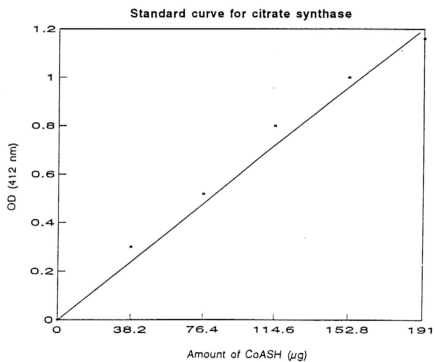
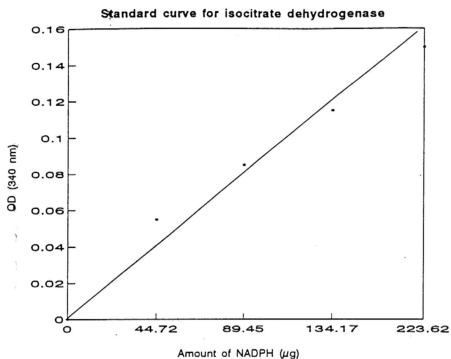
(ii) Protein

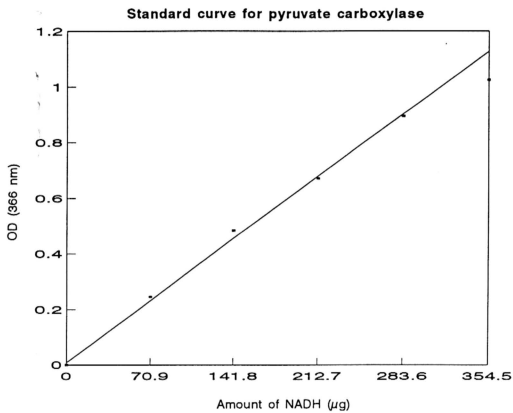
The samples were diluted approximately to 1.0 ml with distilled water. To this, 1.0 ml of Lowry reagent was added and mixed well. The mixture was allowed to stand at room temperature for 20 min. With rapid and immediate mixing, 0.5 ml Colin and Ciocalteu's Phenol reagent was added and the colour was allowed to develop for 30 min. Absorbance (OD) was measured at 625 nm. Protein was determined against a standard curve prepared with BSA at concentration of 80 to 400 $\mu\text{g/ml}$.

APPENDIX IV









Appendix V

(a) Growth Biomass

ANOVA of biomass (Dry Wt.) of subspecies of *B. thuringesis* at 2 1/2 hr growth in culture broth.

Subsps.	Average	Varian	Subsps.	Average	Varian
IPT BT 6	0.467	1.9E-01	IPT BT 16	0.116	6.7E-07
Florbac	0.112	3.3E-06	IMR BT 8	0.035	2.0E-06
IPT BT 15	0.091	1.3E-06	IMR BT 16	0.039	2.0E-0.6
Grand average				0.1433	
Sum square between group				0.527	
Degree of freedom				5	
Average square within group (ABG)				0.105	
Sum square within group				0.573126	
Degree of freedom				18	
Average square within group (AWG)				0.03184	
F-value (5, 18 at 5% probability table)				2.77	
F-test (calculated from ABG/AWG)				3.312763	
Conclusion: The difference among treatments are statistically significant					
AWG				0.03184	
1/ni + 1/nj				0.500	
Standard error (S.E.)				0.126175	
t-value at 5% probability				2.101	

Statistically difference between biomass at 2 1/2 hours

		Ave. Diff.	Confident interval for the difference		Conclusion
			Value1	Value2	
IPT BT 6	IPT Bt 16	0.351	0.09	0.62	significantly different
	Florbac	0.355	0.09	0.62	significantly different
	IPT Bt 15	0.376	0.11	0.64	significantly different
	IPT Bt 16	0.428	0.16	0.69	significantly different
	IMR 8	0.432	0.17	0.70	significantly different
Florbac	IPT Bt 15	0.021	-0.24	0.29	Not significantly different
	IPT Bt 16	0.073	-0.19	0.34	Not significantly different
	IMR 8	0.077	-0.19	0.34	Not significantly different
IPT BT 15	IMR Bt 16	0.052	-0.21	0.32	Not significantly different
	IMR 8	0.056	-0.21	0.32	Not significantly different
IPT BT 16	Florbac	0.004	-0.26	0.27	Not significantly different
	IPT Bt 15	0.025	-0.24	0.29	Not significantly different
	IPT Bt 16	0.077	-0.19	0.34	Not significantly different
	IMR 80.	0.081	-0.18	0.35	Not significantly different
IMR BT 16	IMR 8	0.004	-0.26	0.27	Not significantly different

(b) Reducing Sugar

ANOVA of reducing sugar values of subspecies of *B. thuringesis* at 2 1/2 hr growth in culture broth.

Subsps.	Average	Varian	Subsps.	Average	Varian
IPT Bt 6	1083.30	0.067	IPT Bt 16	1116.70	0.387
Florbac	966.70	0.127	IMR Bt 8	1050.00	3.333
IPT Bt 15	866.70	0.020	IMR Bt 16	950.00	4.667
Grand average				1.005.567	
Sum square between group				176999	
Degree of freedom				5	
Average square within group (ABG)				35400	
Sum square within group				025.803	
Degree of freedom				18	
Average square within group (AWG)				1.4335	
F-value (5, 18 at 5% probability table)				2.77	
F-test (calculated from ABG/AWG)				24694.6	
Conclusion: The difference among treatments are statistically significant					
AWG				1.4335	
1/ni + 1/nj				0.500	
Standard error (S.E.)				0.846611	
t-value at 5% probability				2.101	

Statistically difference at 2 1/2 hours

		Ave. Diff.	Confident interval for the difference		Conclusion
			Value1	Value2	
IPT Bt 6	IMR Bt 8	33.300	31.51	35.08	significantly different
	Florbac	116.600	114.82	118.38	significantly different
	IMR Bt 16	133.300	131.52	135.08	significantly different
Florbac	IPT Bt 15	216.600	214.82	218.38	significantly different
	IMR Bt 16	16.700	14.92	18.48	significantly different
	IPT Bt 15	100.000	98.22	101.78	significantly different
IPT Bt 16	IPT Bt 6	33.400	31.62	35.18	significantly different
	IMR Bt 8	66.700	64.92	68.48	significantly different
	Florbac	150.000	148.22	151.78	significantly different
	IMR Bt 16	166.700	164.92	168.48	significantly different
IMR Bt 8	IMR Bt 15	250.000	248.22	251.78	significantly different
	Florbac	83.300	81.52	85.08	significantly different
	IMR Bt 16	100.000	98.22	101.78	significantly different
IMR Bt 16	IPT Bt 15	183.300	181.52	185.08	significantly different
	IPT Bt 15	83.300	81.52	85.08	significantly different

(c) pH

ANOVA of pH values of subspecies of *B. thuringensis* at 2 1/2 hr growth in culture broth.

Subsps.	Average	Varian	Subsps.	Average	Varian
IPT Bt 6	6.96	3.3E-05	IPT Bt 16	6.93	6.7E-05
Florbac	6.95	2.3E-04	IMR Bt 8	6.99	6.7E-05
IPT Bt 15	6.97	6.7E-05	IMR Bt 16	6.97	3.3E-04
Grand average			6.9617		
Sum square between group			0.008		
Degree of freedom			5		
Average square within group (ABG)			0.002		
Sum square within group			0.002374		
Degree of freedom			18		
Average square within group (AWG)			0.000132		
F-value (5, 18 at 5% probability table)			2.77		
F-test (calculated from ABG/AWG)			12.63584		
Conclusion: The difference among treatments are statistically significant					
AWG			0.000132		
1/ni + 1/nj			0.500		
Standard error (S.E.)			0.008121		
t-value at 5% probability			2.101		

Statistically difference at 2 1/2 hours

	Ave. Diff.	Confident interval for the difference		Conclusion
		Value1	Value2	
IPT Bt 6	Florbac	0.010	-0.01 0.03	Not significantly different
	IPT Bt 16	0.030	0.01 0.05	significantly different
Florbac	IPT Bt 16	0.020	0.00 0.04	significantly different
	IMR Bt 16	0.000	-0.02 0.02	Not significantly different
IPT Bt 15	IPT Bt 6	0.010	-0.01 0.03	Not significantly different
	Florbac	0.020	0.00 0.04	significantly different
	IPT Bt 16	0.040	0.02 0.06	significantly different
IPT Bt 16	IPT Bt 6	0.010	-0.01 0.03	Not significantly different
	Florbac	0.020	0.00 0.04	significantly different
	IPT Bt 16	0.040	0.02 0.06	significantly different
IMR Bt 8	IPT Bt 15	0.020	0.00 0.04	significantly different
	IMR Bt 16	0.020	0.00 0.04	significantly different
	IPT Bt 6	0.030	0.01 0.05	significantly different
	Florbac	0.040	0.02 0.06	significantly different
	IPT Bt 16	0.060	0.04 0.08	significantly different

(d) Protease

Anova of protease between subspecies of *B. thuringesis* after 2 1/2 hr of growth in culture broth.

Replic.	Florbac	IPTBt6	IPTBt15	IPTBt16	IMRBt8	IMRBt16
1	0	0	115.94	0	123.71	152.38
2	0	0	114.83	0	103.09	160.38
3	0	0	105.26	0	103.63	151.66
4	0	0	96.62	0	112.82	156.40
size (ni)	4	4	4	4	4	4
average (xi)	0	0	108.16	0	110.81	155.20
S.D. (Si)	0.00	0.00	9.07	0.00	9.69	4.03
Var. (Vi)	0.00	0.00	82.23	0.00	93.88	16.24
Grand average (X) =	62.36					
(X-xi) ²	3889.20	3889.20	2097.69	3889.20	2347.35	8619.33

Sum square between group	98927.88
Degree of freedom	5
Mean square between group	19785.58
Sum square within group	577.05
Degree of freedom	18
Mean square between group	32.06
F-test	617.17
F-critical value at 5% probability (5,18)	2.77
F-test > F-critical value, thus reject Null hypothesis	
Average square within group	32.06
1/ni+1/nj	5.00
Standard error (S.E.)	4.00
t-value	2.10
S.E. x t-value	8.41

Confident interval values for each average difference specific activity at 2 1/2 hrs.

IMR Bt 16 - IMR Bt 8 = 36 - 53	significantly diff. from each other
IMR Bt 16 - IPT Bt 15 = 38 - 55	significantly diff. from each other
IMR Bt 16 - IPT Bt 6 = 147 - 163	significantly diff. from each other
IMR Bt 16 - Florbac = 147 - 163	significantly diff. from each other
IMR Bt 16 - IPT Bt 16 = 147 - 163	significantly diff. from each other
IPT Bt 6 - IPT Bt 15 = -6.0 - 11.0	not significantly diff. from each other
IPT Bt 6 - IPT Bt 6 = 102 - 119	significantly diff. from each other
IPT Bt 6 - Florbac = 102 - 119	significantly diff. from each other
IPT Bt 6 - IPT Bt 16 = 102 - 119	significantly diff. from each other
IPT Bt 15 - IPT Bt 6 = 100 - 117	significantly diff. from each other
IPT Bt 15 - Florbac = 100 - 117	significantly diff. from each other
IPT Bt 15 - IPT Bt 16 = 100 - 117	significantly diff. from each other
IPT Bt 6 - Florbac = -8.0 - 8.0	not significantly diff. from each other
IPT Bt 6 - IPT Bt 16 = -8.0 - 8.0	not significantly diff. from each other
Florbac - IPT Bt 16 = -8.0 - 8.0	significantly diff. from each other

(e) Alkaline Phosphatase

Anova of alkaline phosphatase between subspecies of *B. thuringesis* after 2 1/2 hr of growth in culture broth.

Replic.	Florbac	IPTBt6	IPTBt15	IPTBt16	IMRBt8	IMRBt16
1	10.7	9.7	10.4	8.9	8.9	8.2
2	11.4	7.8	11.7	9.5	10.4	8.1
3	11.6	8.4	10.7	8.9	9.3	6.8
4	10.2	9.0	11.5	9.5	10.0	9.5
size (ni)	4	4	4	4	4	4
average (xi)	10.98	8.73	11.06	9.20	9.63	8.18
S.D. (Si)	0.65	0.80	0.61	0.37	0.66	1.11
Var. (Vi)	0.42	0.65	0.38	0.14	0.43	1.24
Grand average (X) =	9.63					
(X-xi) ²	1.82	0.82	2.05	0.19	0.00	2.11

Sum square between group	27.91
Degree of freedom	5
Mean square between group	5.58
Sum square within group	9.77
Degree of freedom	18
Mean square within group	0.54
F-test	10.29
F-critical value at 5% probability (5,18)	2.77
F-test > F-critical value, thus reject Null hypothesis	
Average square within group	0.540
1/ni+1/nj	0.500
Standard error (S.E.)	0.520
t-value	2.101
S.E. x t-value	1.092

Confident interval values for each average difference specific activity at 2 1/2 hrs.

IPT Bt 15 - Florbac	= -1.0 - 1.2	not significantly diff. from each other
IPT Bt 15 - IMR Bt 8	= 0.3 - 2.5	significantly diff. from each other
IPT Bt 15 - IPT Bt 16	= 0.8 - 3.0	significantly diff. from each other
IPT Bt 15 - IPT Bt 6	= 1.2 - 3.4	significantly diff. from each other
IPT Bt 15 - IMR Bt 16	= 1.8 - 4.0	significantly diff. from each other
Florbac - IPT Bt 8	= 0.3 - 2.4	significantly diff. from each other
Florbac - IPT Bt 16	= 0.7 - 2.9	significantly diff. from each other
Florbac - IPT Bt 6	= 1.2 - 3.3	significantly diff. from each other
Florbac - IMR Bt 16	= 1.7 - 3.9	significantly diff. from each other
IMR Bt 8 - IPT Bt 16	= -0.7 - 1.5	not significantly diff. from each other
IMR Bt 8 - IPT Bt 6	= -0.2 - 2.0	not significantly diff. from each other
IMR Bt 8 - IMR Bt 16	= 0.4 - 3.9	significantly diff. from each other
IPTBt 16 - IPT Bt 6	= -0.7 - 1.6	not significantly diff. from each other
IPT Bt 16 - IMR Bt 16	= -0.1 - 2.1	not significantly diff. from each other
IPT Bt 6 - IMR Bt 16	= -1.0 - 1.3	not significantly diff. from each other

(f) Pyruvate carboxylase

Anova of pyruvate carboxylase between subspecies of *B. thuringesis* after 2 1/2 hr of growth in culture broth.

Replic.	Florbac	IPTBt6	IPTBt15	IPTBt16	IMRBt8	IMRBt16
1	2.23	0.00	0.00	3.79	0.00	5.00
2	2.27	0.00	0.00	5.66	0.00	6.84
3	2.21	0.00	0.00	4.29	0.00	5.92
4	2.70	0.00	0.00	5.21	0.00	5.92
size (ni)	4	4	4	4	4	4
average (xi)	2.463	0.00	0.00	4.738	0.00	5.922
S.D. (Si)	0.285	0.00	0.00	0.852	0.00	0.751
Var. (Vi)	0.081	0.00	0.00	0.726	0.00	0.564
Grand average (X) =	2.19					
(X-xi) ²	0.076	4.784	4.784	6.505	4.784	13.949

Sum square between group	139.53
Degree of freedom	5
Mean square between group	27.91
Sum square within group	4.11
Degree of freedom	18
Mean square within group	0.23
F-test	122.14
F-critical value at 5% probability (5,18)	2.77
F-test > F-critical value, thus reject Null hypothesis	
Average square within group	0.230
1/ni+1/nj	0.500
Standard error (S.E.)	0.339
t-value	2.101
S.E. x t-value	0.712

Confident interval values for each average difference specific activity at 2 1/2 hrs.

IMR Bt 16 - IPT Bt 15	= 0.5 - 1.9	significantly diff. from each other
IMR Bt 16 - Florbac	= 2.8 - 4.2	significantly diff. from each other
IMR Bt 16 - IPT Bt 6	= 5.2 - 6.6	significantly diff. from each other
IMR Bt 16 - IPT Bt 16	= 5.2 - 6.6	significantly diff. from each other
IMR Bt 16 - IMR Bt 8	= 5.2 - 6.6	significantly diff. from each other
IPT Bt 15 - Florbac	= 1.6 - 3.0	significantly diff. from each other
IPT Bt 15 - IPT Bt 6	= 4.0 - 5.4	significantly diff. from each other
IPT Bt 15 - IMR Bt 16	= 4.0 - 5.4	significantly diff. from each other
IPT Bt 15 - IMR Bt 8	= 4.0 - 5.4	significantly diff. from each other
Florbac - IPT Bt 6	= 1.8 - 3.2	significantly diff. from each other
Florbac - IPT Bt 16	= 1.8 - 3.2	significantly diff. from each other
Florbac - IMR Bt 15	= 1.8 - 3.2	significantly diff. from each other
IPT Bt 6 - IPT Bt 16	= -0.7 - 0.7	not significantly diff. from each other
IPT Bt 16 - IMR Bt 8	= -0.7 - 0.7	not significantly diff. from each other
IPT Bt 16 - IMR Bt 8	= -0.7 - 0.7	not significantly diff. from each other

(g) Isocitrate dehydrogenase

Anova of isocitrate dehydrogenase between subspecies of *B. thuringesis* after 2 1/2 hr of growth in culture broth.

Replic.	Florbac	IPTBt6	IPTBt15	IPTBt16	IMRBt8	IMR Bt16
1	0.003	0.001	0.002	0.002	0.007	0.000
2	0.003	0.001	0.002	0.002	0.006	0.000
3	0.003	0.001	0.003	0.002	0.007	0.000
4	0.004	0.001	0.003	0.002	0.008	0.000
size (ni)	4	4	4	4	4	4
average (xi)	0.0033	0.0010	0.0025	0.0024	0.0069	0.0000
S.D. (Si)	0.0002	0.0001	0.0002	0.0001	0.0006	0.0000
Var. (Vi)	5.82E-08	1.80E-08	2.48E-08	1.09E-08	3.90E-07	0.00E+00
Grand average (X)	= 2.68E-03					
(X-xi) ²	3.91E-07	2.98E-06	1.72E-08	1.70E-07	1.79E-05	7.18E-06

Sum square between group 1.14E-04

Degree of freedom 5

Mean square between group 2.29E-05

Sum square within group 1.51E-06

Degree of freedom 18

Mean square within group 8.37E-08

F-test 273.50

F-critical value at 5% probability (5,18) 2.77

F-test > F-critical value, thus reject Null hypothesis

Average square within group 8.37E-08

1/ni+1/nj 0.500

Standard error (S.E.) 2.05E-04

t-value 2.101

S.E. x t-value 4.20E-04

Confident interval values for each average difference specific activity at 2 1/2 hrs.

IPT Bt 15 - Florbac	= (3.2 - 0.4)E-3	significantly diff. from each other
IPT Bt 15 - IMR Bt 8	= (3.9 - 4.8)E-3	significantly diff. from each other
IPT Bt 15 - IPT Bt 16	= (4.1 - 5.0)E-3	significantly diff. from each other
IPT Bt 15 - IPT Bt 6	= (5.5 - 6.4)E-3	significantly diff. from each other
IPT Bt 15 - IMR Bt 16	= (6.5 - 7.3)E-3	significantly diff. from each other
Florbac - IPT Bt 8	= (3.2 - 11.8)E-4	significantly diff. from each other
Florbac - IPT Bt 16	= (5.2 - 13.8)E-4	significantly diff. from each other
Florbac - IPT Bt 6	= (1.9 - 2.8)E-3	significantly diff. from each other
Florbac - IMR Bt 16	= (2.8 - 3.7)E-3	significantly diff. from each other
IMR Bt 8 - IPT Bt 16	= (-2.3 - 6.3)E-4	not significantly diff. from each other
IMR Bt 8 - IPT Bt 6	= (1.2 - 2.0)E-4	significantly diff. from each other
IMR Bt 8 - IMR Bt 16	= (2.0 - 2.9)E-3	significantly diff. from each other
IPT Bt 16 - IPT Bt 6	= (9.7 - 1.8)E-3	significantly diff. from each other
IPT Bt 16 - IMR Bt 16	= (1.9 - 2.8)E-3	significantly diff. from each other
IPT Bt 6 - IMR Bt 16	= (5.2 - 13.8)E-4	significantly diff. from each other

(h) Citrate Synthase

Anova of citrate synthase between subspecies of *B. thuringesis* after 2 1/2 hr of growth in culture broth.

Replic.	Florbac	IPtBt6	IPtBt15	IPtBt16	IMRbt8	IMR Bt16
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
size (ni)	4	4	4	4	4	4
average (xi)	0	0	0	0	0	0
S.D. (Si)	0	0	0	0	0	0
Var. (Vi)	0	0	0	0	0	0
Grand average (X) =	0					
(X-xi)^2	0	0	0	0	0	0

Sum square between group	0.00
Degree of freedom	5
Mean square between group	0.00
Sum square within group	0.00
Degree of freedom	18
Mean square within group	0.00
F-test	#DIV/0!
F-critical value at 5% probability (5,18)	2.77

APPENDIX VI

Components of the biphasic system used for the purification of spore-crystal suspension

The components of the Upper phase and the Lower phase in the biphasic separation system used for the purification of spore-crystal complex (Goodman *et al.*, 1967).

Lower phase

Sodium dextran sulfate 500 334 ml of 20% (w/v) solution

Polyethylene glycol 6000 234 ml 20% (w/v) solution

Sodium chloride 100 ml of 3M solution

Spore-crystal suspension

Made up to 1L with distilled water

Upper phase

Sodium dextran sulfate 500 1.8 g

Polyethylene glycol 6000 421.8 g

Sodium chloride 105.0 g

Dissolved in 6L of distilled water

APPENDIX VII

Protein standards for electrophoresis

Proteins used as standards for SDS-PAGE and for the determination of their respective molecular weights:

Protein	Mol. weight (Dalton)
Aprotinin	6500
Lysozyme	18,500
Triosephosphate isomerase	26,600
Soybean trypsin inhibitor	27,500
Carbonic anhydrase	32,500
Lactic dehydrogenase	36,500
Fumarase	48,500
Ovalbumin	49,500
Pyruvic kinase	58,500
BSA	80,000
Fructose-6-phosphate kinase	84,000
Phosphorylase B	106,000
β -Galactosidase	116,000
α_2 -Macroglobulin	180,000

Appendix VIII

Molecular weight of standard proteins (Mild Range) used for electrophoresis

