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APPENDICES

Appendix A

Protein concentration is determined by Coomassie brilliant blue G-250 method (Bradford, 1976).

Coomassie brilliant blue G-250	100 mg
95 % ethanol	50 ml
85 % (w/v) phosphoric acid	100 ml

made volume to 1000 ml with distilled water.

An aliquot of 100 µl of protein solution was added to 1 ml of Coomassie brilliant blue G-250 solution for 1-10 µg protein assay. The absorbance at 595 nm was measured after 2 minutes and before 1 hr of incubation. Bovine serum albumin was used as standard protein.

Appendix B

Preparation for polyacrylamide gel electrophoresis.

Stock solution for ND-PAGE

a) 30 % acrylamide solution

acrylamide	30.3 g
bis-acrylamide	0.8 g

adjusted volume to 100 ml with distilled water.

b) Solution B

Tris	18.2 g

adjusted pH to 8.8 with HCl and adjusted volume to 100 ml with distilled water.

c) Solution C

Tris	6.0 g

adjusted pH to 6.8 with HCl and adjusted volume to 100 ml with distilled water.

d) Electrophoresis buffer

Tris	3.0 g
Glycine	14.4 g

adjusted pH to 8.8 with HCl and adjusted volume to 1000 ml with distilled water.

e) Smple buffer

Samples buffer contain 15 % (w/v) sucrose and 0.01 % (w/v) bromophenol blue.

Praparation for ND-PAGE

12 % separating gel

30 % acrylamide solution	4.0 ml
Solution B	2.5 ml
distilled water	3.5 ml
10 % (w/v) ammonium persulfate	50.0 µl
TEMED	10.0 µl

5 % stacking gel

30 % acrylamide solution	0.67 ml
Solution C	1.0 ml
distilled water	2.3 ml
10 % (w/v) ammonium persulfate	30.0 µl
TEMED	10.0 µl

Stock solution for SDS-PAGE

a) 30 % acrylamide solution

acrylamide	30.3 g
bis-acrylamide	0.8 g

adjusted volume to 100 ml with distilled water.

b) Solution B

Tris	18.2 g
10 % (w/v) SDS	4.0 ml

adjusted pH to 8.8 with HCl and adjusted volume to 100 ml with distilled water.

c) Solution C

Tris	6.0 g
10 % (w/v) SDS	4.0 ml

adjusted pH to 6.8 with HCl and adjusted volume to 100 ml with distilled water.

d) Electrophoresis buffer

Tris	3.0 g
Glycine	14.4 g
SDS	1.0 g

adjusted pH to 8.8 with HCl and adjusted volume to 1000 ml with distilled water.

e) Smple buffer

Samples buffer contain 15 % (w/v) sucrose and 2.5 % (w/v) SDS in 125 mM Tris-HCl (pH 6.7), 0.01 % (w/v) bromophenol blue with β-mercaptoethanol for determining molecular weight and without β-mercaptoethanol for detected chitinase activity.

Praparation for SDS-PAGE

12 % separating gel

30 % acrylamide solution	4.0 ml
Solution B	2.5 ml
distilled water	3.5 ml
10 % (w/v) ammonium persulfate	50.0 µl
TEMED	10.0 µl

5 % stacking gel

30 % acrylamide solution	0.67 ml
Solution C	1.0 ml
distilled water	2.3 ml
10 % (w/v) ammonium persulfate	30.0 µl
TEMED	10.0 µl

Staining solution

Coomassie blue R	1.0 g
methanol	450 ml
glacial acetic acid	100 ml
distilled water	450 ml

Destaining solution

methanol	100 ml
glacial acetic acid	100 ml
distill water	800 ml

Appendix C

Preparation of isoelectric focusing polyacrylamide gel.

The gel was set with the following compositions :-

reagent	vol. (ml)
30% acrylamide	0.9
1% bis-acrylamide	1.25
Ampholine	0.243
distilled water	1.39
50% sucrose	1.186
0.02M ammonium persulfate (μl)	39.5

After the run, the gel was stained and destained with the following solutions.

Staining solution

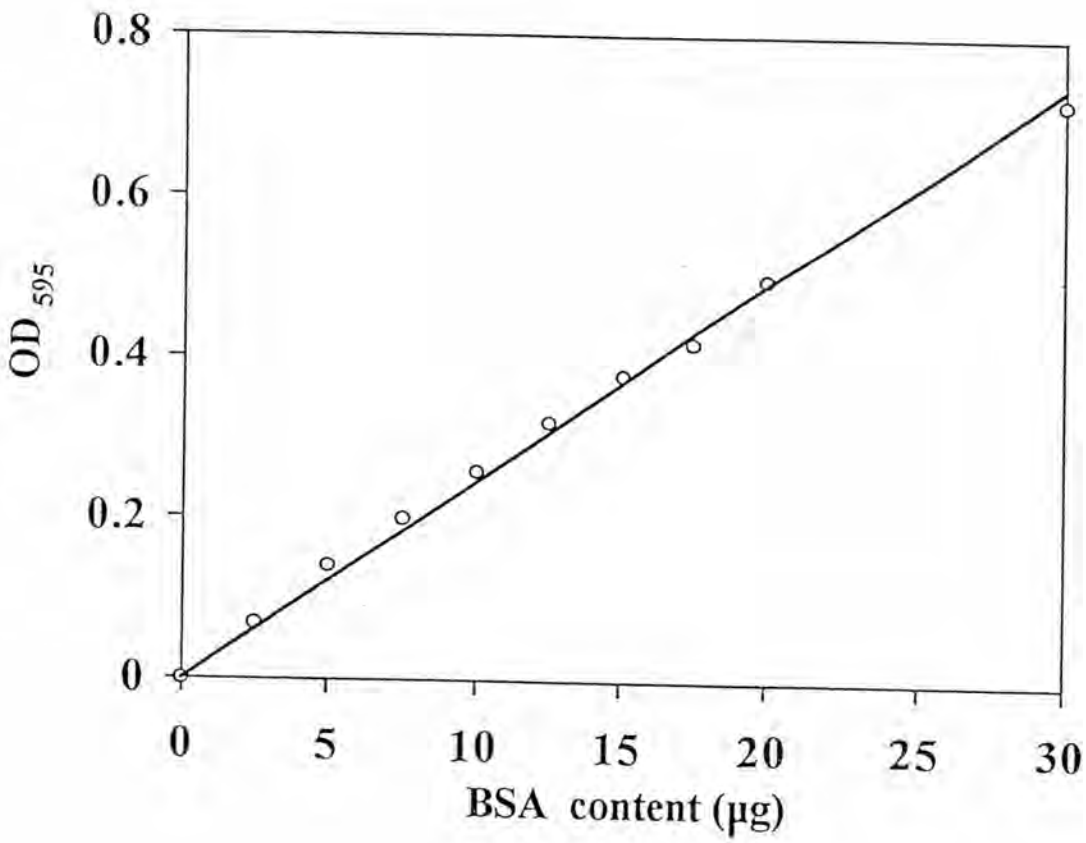
27% ethanol, 10% acetic acid, 0.04% Coomassie brilliant blue R 250 and 0.5% $CuSO_4$

Destaining solution

Composed of 12% (v/v) ethanol, 7% (v/v) acetic acid and 0.5% (w/v) $CuSO_4$. The gel immersed in a few changes of destaining solution until the background was as clear as possible with gentle agitation.

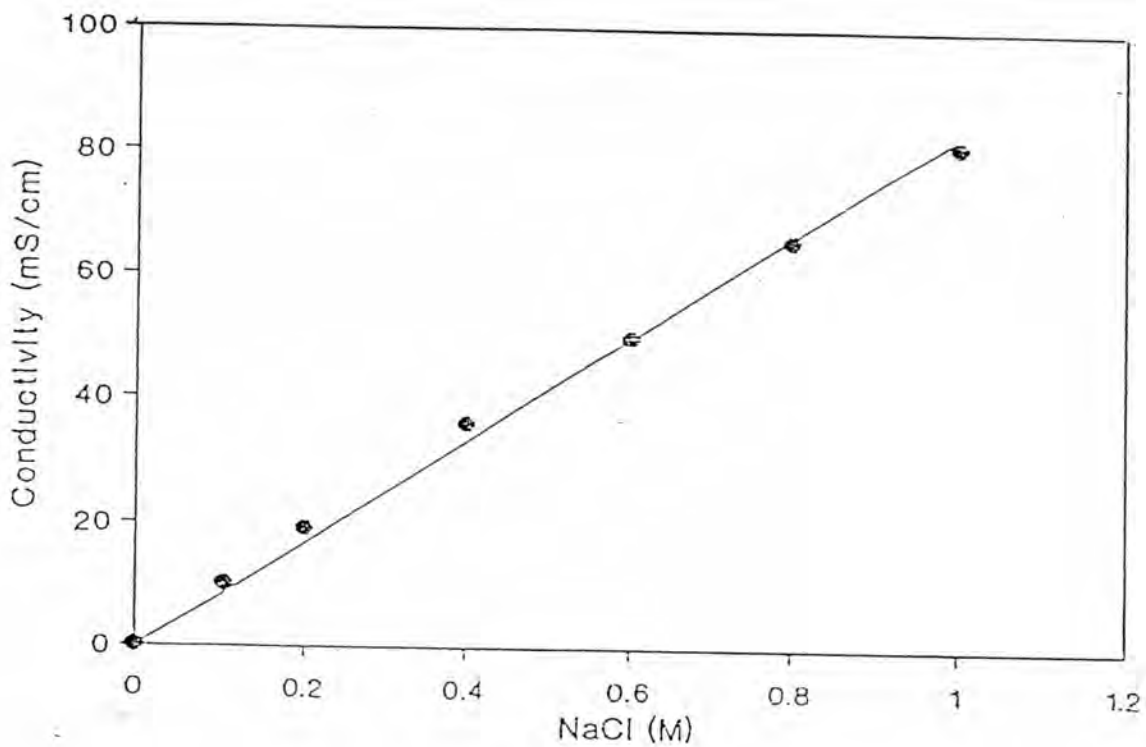
Appendix D

Standard curve of BSA by bradford method



Appendix E

Standard curve of conductivity against NaCl concentration



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