Appendices

APPENDICES

APPENDIX I

COMPOSITION OF LURIA BERTANI (LB) MEDIA

Composition	(g/L)
Tryptone	10g
Yeast extract	5g
NaCl	10g

APPENDIX II

COMPOSITION OF MINIMAL SALT MEDIUM

Composition	(g/L)
Yeast extract	5g
Glucose	1g
Magnesium sulphate	1.5g
Potassium Hydrogen sulphate	0.8g
Potassium dihydrogen sulphate	0.4g
Sodium chloride	30g
pH	7.5g

APPENDIX III

BIOMASS ESTIMATION

(Sampoorna Laxmi, M *et al.*, 2010)

The total protein estimation , the media was centrifuged at 3000 rpm for 15 min at 4°C. The supernatant was discarded and the pellet was washed with distilled water and resuspended in 100 mM phosphate buffer, pH-6.8. This was solicited for 2 min and centrifuged again at 10000 rpm for 15 min at 4°C. The supernatant was collected and used for protein estimation by Lowry method.

APPENDIX IV

ESTIMATION OF LACCASE USIN G ABTS AS SUBSTRATE

(Childs &Bardsley, 1975)

Principle

Laccase activity in the sample was spectrophotometrically determined bymonitoring the rate of product (dark green colour) formation due to theenzymatic oxidation of ABTS.

1 mL of cuvette, the following components were added;

0.5 mL of 2 mM ABTS prepared in buffer of desired pH range + 0.05 to 0.5 mL of enzyme (to be tested) was added, the same buffer in which the ABTS was prepared in, was used to make up the total volume to 1 mL

The kinetic reaction was spectrophotometrically measured at 405 nm for 1 min at the desired temperature, as an increase in absorbance. The blank contained all the assay constituents except the active enzyme, buffer or heat inactivated enzyme was used in its place.

Calculation:

$$\text{Laccase (U L-1)} = \frac{[QA405 * \text{total vol} * \text{dilution factor} * 106]}{[\epsilon ABTS * \text{sample vol}]}$$

Where;

QA405 = rate of reaction i.e. final abs - initial abs ÷ time (min)

ϵABTS = molar extinction coefficient of the radical-cation ABTS (35000)

Total vol = total volume of reaction mixture (mL)

Sample vol = volume of enzyme used (mL)

Laccase was expressed as enzyme units per liter i.e. U L-1 or (μmol min-1) L-1, where one enzyme unit is expressed as μmol of the product formed per minute.

APPENDIX V

ESTIMATION OF PROTEIN

(Lowry *et al.*, 1951)

Principle

The blue colour developed by the reduction of phosphomolybdicphosphotungstic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with alkaline cupric tartarate are measured in the Lowry's method.

Reagents

1. Solution A: 1% Copper sulphate
2. Solution B: 2% Sodium potassium tartarate

3. Solution C: 2% Sodium carbonate in 0.1N sodium hydroxide
4. Solution D: Mixed just before use, 1ml of solution A, 1ml of Solution B and 100ml of Solution C.
5. Solution E: 1N Folinciocalteau reagent (Mixed equal volumes of commercially available reagent and distilled water just prior to use). Stored protected from light.

Standard Bovine Serum Albumin (BSA): 50 mg BSA in 50 ml of 0.1N NaOH. Diluted 1:10 for working standard.

Procedure

Aliquots of standard protein solution (50-1000µg) were taken and the enzyme samples were made upto 1ml with 0.1N NaOH. Shook well to treat the protein with alkali. Added 1ml of Solution D, mixed well and incubated at 37°C for 3 mins. Added 0.1ml of Solution E to each tube, mixed well and incubated at 37°C for 3 mins. Read the colour developed at 670nm against a reagent blank. Fit a linear regression in a scientific calculator and read the protein concentration in the aliquot taken.

Appendix VI

Composition of Trace element solution (L)

Boric acid	-	0.3g
Calcium Chloride	-	10mg
Cobaltous chloride	-	0.2g
Zinc Sulphate	-	0.1g
Manganese Chloride	-	30mg
Sodium Molybdate	-	30mg
Copper Sulphate	-	10mg