Appendix
Appendix 1

1. Reagents for Poly Acrylamide Gel Electrophoresis

(1) Stock acrylamide solution (30:0.8)
Acrylamide (30 %) - 60.0 g
Bis-acrylamide (0.8 %) - 1.6 g
Distilled water (DW) - 200.0 ml
Stored at a temperature of 4°C in amber colored bottle.

(2) Stacking gel buffer stock (0.5 M Tris-HCl, pH 6.8)
Tris buffer - 6 g in 40 ml DW
Titrated to pH 6.8 with 1M HCl and made up to 100ml with DW. Filtered with Whatman No.1 filter paper and stored at 4°C.

(3) Resolving gel buffer stock (3MTris-HCl, pH 8.8)
Tris buffer - 36.3 g
Titrated to pH 8.8 with 1 M HCl and made up to 100 ml with DW. Filtered with Whatman No.1 filter paper and stored at 4 °C.

(4) Reservoir buffer for SDS-PAGE
Tris buffer - 3.0 g
Glycine - 14.4 g
SDS - 1.0 g
Dissolved and made up to 1L with DW.
Prepared in 10X concentration and stored at 4 °C.

(5) Sample buffers
(a) Sample buffer for non-reductive SDS-PAGE
Tris -HCl (pH6.8) - 0.0625 M
Glycerol - 10 % (v/v)
SDS - 2 %
Bromophenol blue - 0.01 %
(b) Sample buffer for reductive SDS-PAGE
Tris-HCl (pH 6.8) - 0.0625 M
Glycerol - 10 % (v/v)
SDS - 2 %
Dithiothreitol - 0.1 M
Bromophenol blue - 0.01 %
Prepared as 2X concentrations and stored at 4 °C

(6) SDS (10 %) - 1gm in 10 ml DW

(7) Sucrose (40 %) - 4gm in 10 ml DW
(Autoclaved at 121 °C for 15 min and stored at 4 °C)

(8) Protein staining solution
Coomassie Brilliant blue
(0.1 %) - 100 mg
Methanol (40 %) - 40 ml
Glacial acetic acid - 10 ml
DW - 50 ml

(9) Destaining Solution
Methanol (40 %) - 40 ml
Glacial acetic acid (10 %) - 10 ml
DW - 50 ml

(10) Protein marker for SDS-PAGE
Low molecular weight marker mix of (Bangalore Geni) was used. Marker mix was reconstituted in IX sample buffer for SDS-PAGE, boiled for 5 min, and 5 µl of marker was loaded on to the gel. The composition of the marker mix is as given below.

<table>
<thead>
<tr>
<th>Components</th>
<th>MW(M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin, rabbit muscle</td>
<td>205,000</td>
</tr>
<tr>
<td>Phosphorylase b</td>
<td>97,000</td>
</tr>
<tr>
<td>Bovine Serum Albumin</td>
<td>66,000</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>45,000</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>30,000</td>
</tr>
<tr>
<td>Trypsin inhibitor</td>
<td>20,100</td>
</tr>
<tr>
<td>a-Lactalbumin</td>
<td>14,400</td>
</tr>
</tbody>
</table>
2. Preparation of Native PAGE

Gel preparation

Resolving Gel (10 %)
Acrylamide: bis-acrylamide – 10.0 ml
Resolving gel buffer stock – 3.75 ml
DW – 16.25 ml
TEMED - 15μl
Ammonium per sulphate (APS) – 0.15 ml

Stacking gel (2.5 %)
Acrylamide: bis-acrylamide - 1.25 ml
Stacking gel buffer stock - 2.5 ml
DW - 6.15 ml
TEMED - 15 μl
Ammonium per sulphate (APS) - A pinch

3. Sample buffer

Sample buffer- Native PAGE (2X)- 1ml
50 % Sucrose-0.4ml
DW-0.6 ml.
Appendix 2

1. **18srDNA sequence** (Accession number KM516789)

```
GGAAGTTTTGGCTTGGTTTAAGATTAAGCCATGCATGTCTAAGTATAAGCACTTT
ATACTGTAAGACTGGAATTTACTCTCACTATATCTGCAACGGTGGATAGCTAC
CTTACTACATGGAATCCTGTGGAATATTCTAGAAGCTAATACATGCTGAAAACCTCG
ACTTCCGAAGGGGTGTATTTTTATTAGATAAAAAACCAATGGCCTTCGGGGCTCCTT
GGTGAATCATAAATCCACTAAGGAAACAGAATTTGAAAGGACAGTCTGTCAGTAC
CAAATTTCGCCCATCAACTTTCCATGTTAGGATAGTGGCCTACCATGGAACAC
ACGGGTAAAGGGGATTAGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTTC
ACCACATCCAAGGAAAGGACAGCGCCGACAAATACCCCAATTCGACACCGGGA
GGTAGTGACATATATACGATAACGGGCTCTTTGGTCTCGTATATTGGAAATG
GTACAAATCTAATCCACTAAGGAAACAGAATTTGAAAGGACAGTCTGTCAGTAC
ACCCCGCTGATATCAGCAGCAGCTTTTACCTTGGAAGTACCTGTCAGTAC
GACTGGGTATATCAGCAGCAGCTTTTACCTTGGAAGTACCTGTCAGTAC
TGGAAATTTCTCGTGAAGAAGCTACTACTAGCAGGAAACGTCCAGAGGGGGAGAT
GTTTTTATTAATCATAGGAAAGCTCTTTTATGATGCCAGTCAGTCTAGG
AGCAGAGATTATGGTGGATGAGGGTATGAGTCAGGTTC
TGCTGGGAAGATGAATATGACGGTGTTATCGCTGGTGCCCCTGCCTTCCGCTT
TGCTCAGCAGGTTCCACCACGCCTTCCCTGCCACTATCGAACATACCATGGAT
TACTACCCTCTCGAAGTCTGCAAAAAGTTGGTCTCAGTCTGCAAAA
```

2. **Sequencing of the tannase gene of 550bp fragment as given below**

```
CGATGCTACTGGCGTCTCGAGTACGGTGTTGCTGCTGGCTGGGGCCAGATGCGCC
TCGATACGCTCTCCTCAACTAGCAGGAAAGGTGCTCTGCTGCTCCGCTGCTCC
TACACTCAGGAGGTCTGGTCTCCGCTGCTGGCTGGGGCCAGATGCGCC
GTACGGGTAGTTATCTGGTCTGCTCCGCTGCTGGCTGGGGCCAGATGCGCC
AGGAGGTTATCTGGTCTGCTCCGCTGCTGGCTGGGGCCAGATGCGCC
TACACTCAGGAGGTCTGGTCTCCGCTGCTGGCTGGGGCCAGATGCGCC
ATAGACGGGTAGTTATCTGGTCTGCTCCGCTGCTGGCTGGGGCCAGATGCGCC
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3. Proof of Culture deposition

Dr. G.S. Prasad
Senior Principal Scientist

By Courier
28.04.2014

Dear Dr. C. Prabha Kumari,

Your microbial culture has been accepted for deposit in MTCC-IDA under Budapest Treaty. It was assigned MTCC number and preserved in the patent collection of MTCC. The details are as follows,

Taxonomic designation Identification Reference MTCC Number Assigned Date of Accession
Aspergillus niger CEPC - 11 MTCC 5889 20.01.2014

Enclosed here with are the relevant documents of the above strain,

Form BP/4 - Receipt and acceptance of the culture in MTCC
Form BP/9 - Viability statement of the culture

Please acknowledge the receipt of the forms.

Sincerely yours,

(G. S. PRASAD)