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APPENDICES

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX A

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M. T.: 9  ATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGAAGC 68
          |||
M. K.: 1  ATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGTGAAGC 60

M. T.: 69  AGGAGCTTGCTCTTGTGGATCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCC 128
          |||
M. K.: 61  AGGAGCTTGCTCTTGTGGATCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCC 120

M. T.: 129  GGACTCTGGGATAAGCGCTGGAAACGGCGTCTAATACTGGATACGAGTAGCGACCGCATG 188
          |||
M. K.: 121  GGACTCTGGGATAAGCGCTGGAAACGGCGTCTAATACTGGATACGAGTAGCGACCGCATG 180

M. T.: 189  GTCAGCTATTGGAAAGAATTTTCGGTCTGGGATGGGCTCGCGGCCTATCAGCTTGTGGTG 248
          |||
M. K.: 181  GTCAGCTATTGGAAAGAATTTTCGGTCTGGGATGGGCTCGCGGCCTATCAGCTTGTGGTG 240

M. T.: 249  AGGTAATGGCTCACCAAGGCGTCGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACT 308
          |||
M. K.: 241  AGGTAATGGCTCACCAAGGCGTCGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACT 300

M. T.: 309  GGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG 368
          |||
M. K.: 301  GGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG 360

M. T.: 369  GCGCAAGCCTGATGCAGCAACCCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCT 428
          |||
M. K.: 361  GCGCAAGCCTGATGCAGCAACCCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCT 420

M. T.: 429  TTTAGTAGGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAAAAGCGCCGGCTAACTACGT 488
          |||
M. K.: 421  TTTAGTA-GGGAAGAAGCGAAAGTGACGGTACCTGCAGAAAAAGCGCCGGCTAACTACGT 479

M. T.: 489  GCCAGCAGCCCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGA 548
          |||
M. K.: 480  GCCAGCAGCCCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGA 539

M. T.: 549  GCTCGTAGGCGGTTTGTTCGCTCTGCTGTGAAATCTGGGGGCTCAACCCCA-CCTGCAG 607
          |||
M. K.: 540  GCTCGTAGGCGGTTTGTTCGCTCTGCTGTGAAATCTGGGGGCTCAACCTCNAGCCTGCAG 599

M. T.: 608  TGGGTACCGGCAGACTAAAGTGCCGTAGGGGAGATTGGAATCCTGGTGTAAACCGTGGAA 667
          |||
M. K.: 600  TGGGTACCGGCAGACTAGAGTCCGGTAGGGGAGATTGGAATCCTGGTGTAGCGGTGGAA 659

M. T.: 668  TGCCCCAAATTTTCAGGAGGAACACCCGATGGCGAAGGCGGATCTTTGGCCCTAACT 724
          |||
M. K.: 660  TG-CGCAGATATCAGGAGGAACA-CCGATGGCGAAGGCGAGATCTCTGGCCGTA ACT 714

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Figure A1 The 16S rRNA sequence of *Microbacterium* sp. TU005 (E) (M. T), compared with *Microbacterium keratanolyticum* (M. K).

APPENDIX B

Preparation for denaturing polyacrylamide gel electrophoresis

Stock solution

1. 2M Tris-HCl pH8.8

Tris(hydroxymethyl)-aminomethane 24.2 g

Adjust pH to 8.8 and make volume to 100 ml.

2. 1M Tris-HCl pH6.8

Tris(hydroxymethyl)-aminomethane 12.1 g

Adjust pH to 6.8 and make volume to 100 ml.

3. 10% (W/V) SDS

SDS 10.0 g

Adjust volume to 100 ml. The solution will keep at roomtemp

4. 50% Bromophenol blue

Bromophenol blue 100 mg

Adjust volume to 10 ml with distilled water.

5. 50% (V/V) glycerol

100% Glycerol 50 ml , added with 50 ml of distilled water.

6. 1% Glycol chitin

0.01 g of Glycol chitin, dissolved in 1 ml ultrapure water.

Working solution

Solution A (Acrylamide stock solution)

(30%(W/V) acrylamide 0.8% bis-acrylamide)

Acrylamide 29.2 g

N,N'-dimethylene-bis-acrylamide 0.8 g

Adjust volume to 100 ml with distilled water.

Solution B

2M Tris-HCl pH8.8	75.0 ml
10% SDS	4.0 ml
Distilled water	21.0 ml

Solution C

1M Tris-HCl pH6.8	50.0 ml
10% SDS	4.0 ml
Distilled water	46.0 ml

SDS electrophoresis buffer

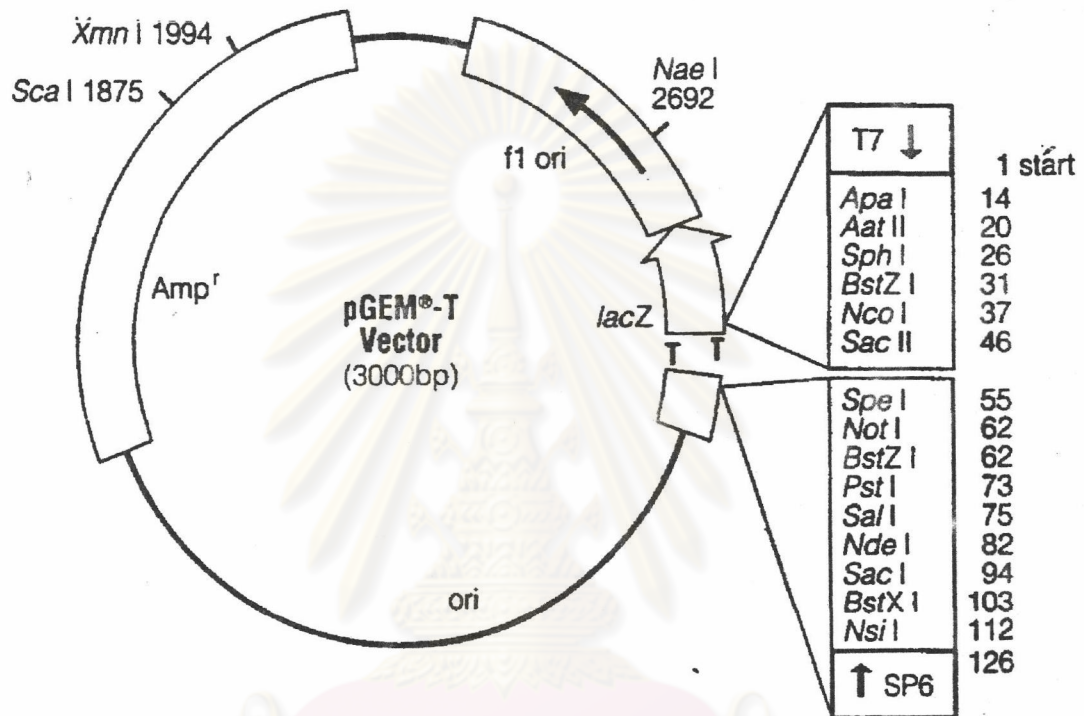
Tris(hydroxymethyl)-aminomethane	3.03 g
Glycine	14.40 g
SDS	1.0 g

Preparation of SDS-PAGE (10% seperating gel) for activity stain.

Reagent	10% Seperating gel	5% Stacking gel
Solution A	6.66 ml	1.34 ml
Solution B	5.0 ml	-
Solution C	-	2.0 ml
10% ammonium persulfate	100 μ l	60 μ l
TEMED	10 μ l	10 μ l
Distilled water	8.03 ml	4.6 ml
1% glycol chitin	200 μ l	-
Total volume	20 ml	8 ml

APPENDIX C

Restriction map of pGEM-T easy vector



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APPENDIX D

Standard curve of N-acetyl-D-glucosamine for chitinolytic enzyme assayed by colorimetric method.

Standard curve for N-acetyl-D-glucosamine (GlcNAc) was made by determining the absorbance value at 420 nm of standard GlcNAc according to the method of schale.

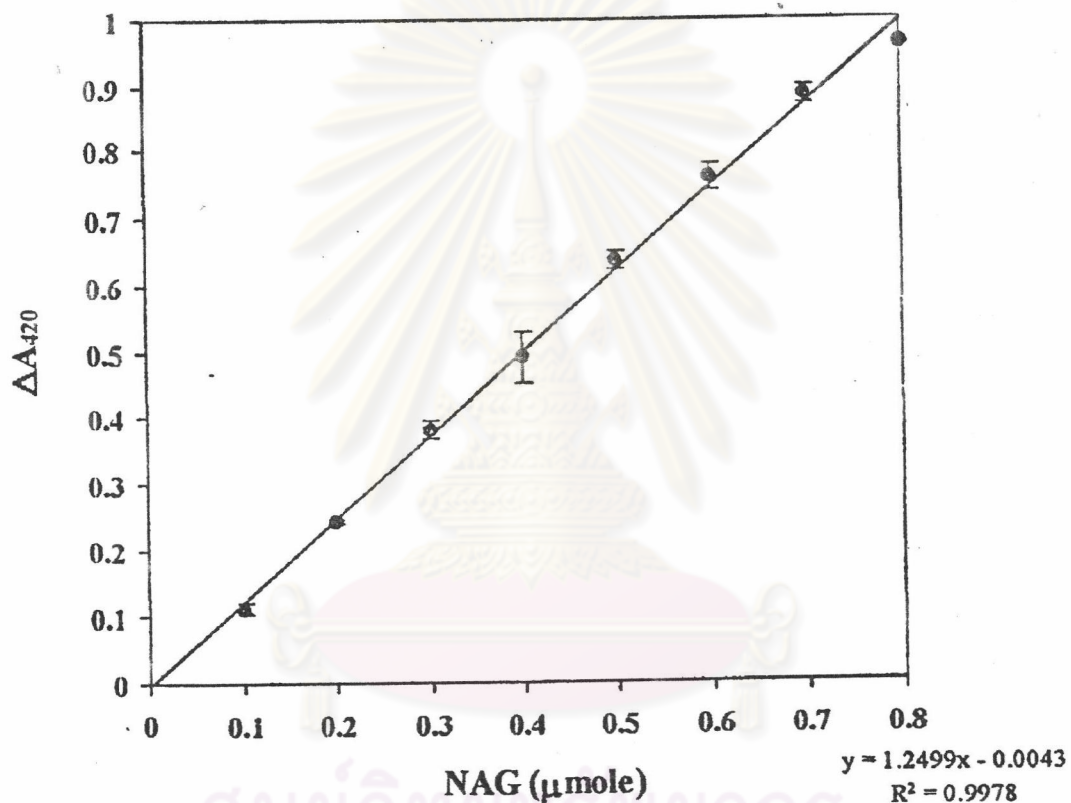


Figure D1 Correlation between final concentration of standard N-acetyl-D-glucosamine and absorbance at 420 nm.

BIOGRAPHY

Miss Janjaras Sermsatanaswadi was born on September 27, 1976 in Bangkok. After she finished Mattayom VI in 1991 from Satriwittaya School, she was enrolled in the Biochemistry, Chulalongkorn University and graduated with the B.Sc. in 1997. She entered the graduated program for M.Sc. in Biochemistry at Chulalongkorn University in 1998.



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